Narrow-leaved Lupine (*Lupinus angustifolius* L.) as Nitrogen Source in Organic Vegetable Production Systems

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Abstract

Green manure legumes represent an important N source in organic farming systems. However, since neither the amount of N_2 fixed nor net N mineralization from soil incorporated legume biomass can be influenced satisfactorily, N availability for following crops does often not match their requirements.

In pot and field experiments the potential of lupines (*Lupinus angustifolius* L.) to be used as flexible and well controllable N source alternative in organic vegetable production was investigated. Maximum net N mineralization ($N_{net\ max}$) from coarsely milled lupine seeds as derived from a first-order kinetics model averaged 57%. Under laboratory conditions, $N_{net\ max}$ was up to 44% higher for lupine seedlings compared to seed meal, which was explained by germination processes causing an initial decrease in lupine C:N ratio. The linear regression relating $N_{net\ max}$ to C:N ratio was in close agreement with that obtained in field experiments with white cabbage, when short-term positive priming effects occurring under field conditions were ignored.

Nitrogen use efficiency (NUE) of cabbage decreased with increasing N availability. As revealed by NUE component analysis, this was exclusively due to an increasing N concentration in cabbage above-ground biomass. Among NUE components, dry matter harvest index was least dependent on N availability but was considered to be affected by thermal time from crop establishment to harvest as well as by agronomic factors controlling early crop growth. The residual effect of lupine amendments on N availability for a subsequent beetroot crop was largely attributed to incremental N in cabbage residues, while potentially late mineralizing lupine seed N did not contribute observably to beetroot N supply.

Comparison of crop rotations including either lupines or differently managed grass-clover swards resulted in N inputs via symbiotic N_2 fixation being largely comparable if grass-clover was mulched and cut sward biomass remained on the field. On average of two experimental years, removal of sward biomass lead to a significant, more than two-fold, increase in N_2 fixation but decreased N availability for subsequent beetroot. Net N mineralization from grass-clover residues within

the year of their incorporation was positively related to the percentage of clover in the mixture, varying with experimental year and sward management.

From potential N leaching losses after legume precrops it is concluded that local production of lupine seeds followed by their reallocation as fertilizer provides a viable N source alternative to mulched grass-clover swards on sites with either low N leaching risk or low to moderate mineralization potential of indigenous soil organic N.

Keywords: Organic vegetable production · *Lupinus angustifolius* · Nitrogen flows

Kurzfassung

In ökologische Fruchtfolgen integrierte Leguminosen spielen eine wichtige Rolle als N-Quelle. Die Anpassung der N-Verfügbarkeit an den Bedarf nachfolgender Gemüsekulturen gestaltet sich jedoch aufgrund der nicht beeinflussbaren und stark variierenden N_2-Fixierungsleistung sowie eines nur unzureichend steuerbaren Mineralisationsverlaufes als problematisch.

Die Möglichkeit einer verbesserten N-Steuerung durch den Anbau von Lupinen (*Lupinus angustifolius* L.) gefolgt von der temporären Lagerung des Kornmaterials und seiner flexiblen sowie gut kalkulierbaren Wiederausbringung als N-Dünger wurde in Freiland- und Gefäßversuchen untersucht. Die mittels einer erweiterten Mitscherlich-Funktion quantifizierte maximale Netto-N-Mineralisation ($N_{net\ max}$) von geschrotetem Lupinenkorn betrug durchschnittlich 57%. Unter kontrollierten Bedingungen wiesen Lupinenkeimlinge im Vergleich zu Lupinenschrot um bis zu 44% höhere Werte für $N_{net\ max}$ auf, was mit einer durch Keimungsprozesse bedingten Absenkung des C:N-Verhältnisses erklärt wurde. Die in Freilandversuchen mit Weißkohl quantifizierte Beziehung zwischen $N_{net\ max}$ und C:N-Verhältnis war in Übereinstimmung mit der im Gefäßversuch ermittelten Beziehung, sofern kurzzeitig auftretende positive Priming-Effekte ignoriert wurden.

Die Stickstoffnutzungseffizienz (NUE) von Weißkohl nahm mit steigender N-Verfügbarkeit ab, was ausschließlich durch eine zunehmende N-Konzentration im oberirdischen Aufwuchs bedingt war. Unterschiede im Harvest Index wurden durch die Variation der N-Verfügbarkeit nur unzureichend erklärt. Es deutet sich an, dass sowohl die Temperatursumme der Vegetationsperiode als auch Wachstumsfaktoren, welche die frühe Pflanzenentwicklung bestimmen, wesentlichen Einfluss auf den Harvest Index und damit auf die NUE haben. Der Einsatz von Lupinenkorn als N-Dünger erhöhte die N-Menge in Kohlernterückständen, was indirekt zu einem positiven Residualeffekt auf das N-Angebot für nachfolgende Rote Bete führte. Direkte Residualeffekte, bedingt durch eine fortschreitende Netto-N-Mineralisation des Kornmaterials, waren nicht in relevanter Größenordnung feststellbar.

Die Gegenüberstellung von Lupinen- und unterschiedlich genutzten Kleegrasbeständen in Fruchtfolgeversuchen ergab einen weitgehend vergleichbaren N-Input durch symbiotische N_2-Fixierung, sofern das Kleegrasgemisch gemulcht wurde und das Schnittgut auf dem Feld verblieb. Die Abfuhr des Schnittguts führte im Mittel von zwei Versuchsjahren zu einer signifikanten Steigerung der N_2-Fixierungsleistung um mehr als das Doppelte, reduzierte jedoch die N-Verfügbarkeit für nachfolgende Rote Bete. Die Netto-N-Mineralisation von im Frühjahr eingearbeiteter Kleegras-Biomasse im Verlauf der Vegetationsperiode nahm mit steigendem Kleeanteil im Kleegrasaufwuchs zu. Der Kleeanteil variierte in Abhängigkeit von Nutzungsform und Versuchsjahr.

Unter Berücksichtigung potentieller N-Verluste durch Auswaschung wird geschlussfolgert, dass das untersuchte Lupinensystem auf Böden mit geringem bis moderatem N-Nachlieferungspotential oder auf Standorten mit geringem Auswaschungsrisiko eine Alternative zu mulchgenutzten Kleegrasbeständen darstellt.

Schlagworte: Ökologische Gemüseproduktion · *Lupinus angustifolius* · Stickstoffflüsse

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Abbreviations and acronyms

C_c	content of clover in above-ground sward dry matter	%
CM	coarse meal	
CR	cabbage residues	
C_t	total C concentration	%
CT	cut grass-clover	
DM	dry matter	
DMc	dry matter concentration	%
DM_{ngs}	non-germinated seed dry matter	g m^{-2}
DM_s	seed dry matter	g m^{-2}
DM_{sc}	seed coat dry matter	g m^{-2}
DM_{sdl}	seedling dry matter	g m^{-2}
DS	dense sowing	
DS-L	dense sowing, long developmental time (field experiment)	
DS-S	dense sowing, short developmental time (field experiment)	
DS-*x*	dense sowing, *x* days of age (pot experiment)	
ET_0	reference evapotranspiration	mm d^{-1}
Et_c	actual evapotranspiration	mm d^{-1}
GR	germination rate	%
k	mineralization rate constant	[°Cd]$^{-1}$
K_c	crop evapotranspiration coefficient	
K_{cb}	basal crop coefficient	
K_e	evaporation coefficient	
LNY	lupine N yield	kg ha^{-1}
MU	mulched grass-clover	
N_{av}	total available N (N supply)	kg ha^{-1}
$N_{av,lup}$	total available N for white cabbage following lupine	kg ha^{-1}

$N_{av,sw}$	total available N for white cabbage following spring wheat	kg ha^{-1}
N_{cr}	cabbage harvest residue N	kg ha^{-1}
N_{fix}	N_2 fixation	kg ha^{-1}
$N_{LEG\ leaf\ lit.}$	lupine leaf litter N	kg ha^{-1}
$N_{LEG\ cut}$	cut grass-clover above-ground N	kg ha^{-1}
$N_{LEG\ root}$	legume crop below-ground N	kg ha^{-1}
$N_{LEG\ seed}$	legume crop seed N	kg ha^{-1}
$N_{LEG\ shoot}$	legume crop above-ground N	kg ha^{-1}
$N_{LEG\ tot}$	total legume crop N	kg ha^{-1}
N_{net}	net N mineralization	%
$N_{net\ max}$	maximum net N mineralization	%
$N_{net,CM}$	coarse meal net N mineralization corrected for lupine N yield	kg ha^{-1}
$N_{net,ow}$	over-winter net N mineralization	kg ha^{-1}
N_{ngs}	non-germinated seed N	g m^{-2}
N_p	total above-ground plant N	kg ha^{-1}
N_{peak}	measure of N priming effect	kg ha^{-1}
$N_{rec\ seedl}$	N recovered in seedling biomass	%
$N_{rec\ soil}$	N recovered in soil as mineral N	%
$N_{rec\ sum}$	sum of N recovered in seedling biomass and in soil as mineral N	%
$N_{REF\ cut}$	cut ryegrass above-ground N	kg ha^{-1}
$N_{REF\ root}$	ryegrass below-ground N	kg ha^{-1}
$N_{REF\ seed}$	ryegrass seed N	kg ha^{-1}
$N_{REF\ shoot}$	ryegrass above-ground N	kg ha^{-1}
$N_{REF\ tot}$	total ryegrass N	kg ha^{-1}
N_{sc}	seed coat N	g m^{-2}
N_{sdl}	seedling N	g m^{-2}
N_t	total N concentration	%
$N_{tot\ max}$	total maximum net N mineralization	%
NUE	N use efficiency	kg kg^{-1}

N_{upt}	net N uptake	%
N_y	crop N yield	kg ha^{-1}
PAR	photosynthetically active radiation	W m^{-2}
P_{dm}	total above-ground plant dry matter	g m^{-2}
PE_{lup}	lupine precrop N effect	kg ha^{-1}
RE_{leg}	legume residual N effect	kg ha^{-1}
$r_{N,CM}$	coarse meal net N mineralization	%
SCP	seed coat proportion	%
SEM	standard error of the mean	
SMN	soil mineral N	
SMN_{LEG}	legume crop soil mineral N	kg ha^{-1}
SMN_{REF}	ryegrass soil mineral N	kg ha^{-1}
SOM	soil organic matter	
$SOMN_{net,leg}$	net N mineralization from soil organic matter following legume precrop	kg ha^{-1}
$SOMN_{net,sw}$	net N mineralization from soil organic matter following spring wheat	kg ha^{-1}
SWC	soil water content	g cm^{-3}
t_{95}	thermal time to 95% of $N_{net\ max}$	°Cd
Y_{dm}	dry matter yield	g m^{-2}
Y_{fm}	fresh matter yield	t ha^{-1}

1 General introduction

1.1 Background

Research in the 20th century has determined the nitrogen (N) requirement of most vegetable crops (Hochmuth 2003) and developed soil testing methods, which allow considering the indigenous soil N supply (Németh et al. 1979; Wehrmann and Scharpf 1979). Nowadays in conventional vegetable production, the use of inorganic N fertilizers allows for a precise and comparatively easy adjustment of N supply to crop requirements, which is in contrast to the present situation in organic production. Nitrogen availability for organically grown vegetable crops depends on the presence and, respectively, on the availability of various organic N sources, including soil organic matter, compost, (green) manure and animal-waste products like bone, horn or feather meal, as well as on their specific mineralization characteristics (Gaskell and Smith 2007; Laber 2003).

As organic vegetable production systems typically lack livestock, animal manure is rarely available as N source (Ulrich et al. 2007). Leguminous green manure crops have the potential to contribute significant amounts of N to subsequent crops and hence play a major role in organic vegetable rotations (Haas et al. 2007; Laber 2007; Thorup-Kristensen 2006; Willumsen and Thorup-Kristensen 2001). Even though the N input via N_2 fixation by legume green manure crops may be substantial, it varies greatly by a number of factors, including legume species, crop age and soil N status. Factors that limit plant growth usually also limit N_2 fixation (Cuttle et al. 2003). Net N mineralization from decomposing green manure crops and thus their N benefit for following vegetable crops depend largely on residue quality, soil moisture and temperature as well as on specific soil factors such as texture and composition of microbial community (Cabrera et al. 2005). Variability in both the amount of N accumulated in green manure biomass and the time course of its net mineralization makes it difficult to predict and particularly to control N availability after ploughing green manure crops. As a consequence, the amount of available N provided by traditional legume green manures, such as alfalfa or clover, either grown in mixture with grass or in pure

stand, does often not match N requirements of following crops (Båth 2000; Crews and Peoples 2005; Harris et al. 2006).

While particularly N demanding organic vegetable crops are frequently undersupplied with N, green manuring does involve a substantial risk of N leaching losses to the environment. In stockless production systems, mulching of grass-clover swards is common practice, since cut herbage is not required for forage use. Nitrogen leaching may occur already during the sward phase from decomposing mulch N (Dreymann 2005) as well as after sward ploughing from an increased N mineralization from soil organic matter being not in synchrony with crop N uptake (Kayser et al. 2009; Stopes et al. 1996).

1.2 Matching N supply with crop demand

Legume green manuring systems which allow for adjusting N availability closely to crop requirements have to allow for uncoupling N availability from the performance of the preceding legume. In traditional green manuring, this is typically restricted by the fact that N derived from N_2 fixation is to a large extent location- and time-bound. Grain legumes, such as faba bean, lupine or pea, may have the potential to provide a flexible N source alternative to the currently predominating use of forage legumes.

During the pod-filling stage, grain legumes remobilize vegetative N, which is translocated to developing seeds (Herdina and Silsbury 1990; Howieson et al. 1998). Later, at crop maturity, a large proportion of total plant N is concentrated in the seed biomass being easily harvestable and well storable. Reallocation of grain legume seeds as organic N fertilizer can be carried out in controllable amounts flexibly in time and space. As already reported by Becker-Dillingen (1929), seeds of white lupine (*Lupinus albus* L.) had been produced on Corsica, France, to be thereafter utilized as N fertilizer in the area of Toscana, Italy. While this practice was discontinued due to the upcoming use of synthetic fertilizers, grain legume seeds have been recently rediscovered as N fertilizer for organic horticulture (Müller and von Fragstein und Niemsdorff 2006a; 2006b; Stadler et al. 2006), where the use of synthetic N fertilizers is banned.

1.3 Objectives and outline of the thesis

The overall purpose of the present work was to examine the potential of lupines to be used as flexible N source in organic field vegetable production. Specific objectives, each covered in a dedicated chapter, were

- to characterize the temporal N mineralization pattern of lupine seeds either applied as coarse meal or intact seeds followed by soil-incorporation of seedlings (Chapter 2),
- to investigate the impact of lupine seed application method on the N use efficiency of white cabbage and to quantify residual effects on a subsequent crop (Chapter 3),
- to compare the ability of lupines, integrated into organic vegetable rotations, to fix N_2 from the atmosphere and to provide this N to succeeding crops with that of common grass-clover swards (Chapter 4).

Each chapter was indented to be published independently of the other two and thus can be read individually.

2 Decomposition of lupine seeds and seedlings as N fertilizer in organic vegetable production

Abstract

Seeds of grain legumes are currently discussed as N fertilizer in organic vegetable production. They can be produced by farmers themselves and applied in well-controlled amounts flexibly in time and space. Most research investigating the N mineralization of grain legume seeds was carried out using coarsely milled seeds. It was hypothesized that seed germination alters the chemical composition leading to a higher N mineralization compared to coarse meal.

In a pot experiment the C:N ratio of lupine seedlings was shown to decrease from initially 8.8 to a minimum of 6.2 within the first two weeks after sowing prior to a re-increase. Maximum net N mineralization ($N_{net\ max}$) as derived from a first-order kinetics model was up to 44% higher for seedlings compared to coarse meal and was linearly related to the altered C:N ratio.

Under field conditions on a loess-derived Typic Hapludalf soil, net N mineralization of incorporated lupine materials showed initial peaks partly exceeding the amount of N added with lupine seeds. These short-term priming effects were found to be more intense for lupine coarse meal than for seedlings. Ignoring mineralization peaks when fitting the first-order kinetics model to the field experimental data, the resulting relationship between $N_{net\ max}$ and C:N ratio was in close agreement with that obtained in the pot experiment. However, net N mineralization in the field was not significantly increased by sowing compared to the application as coarse meal, even if seedlings were incorporated as early as feasible under field conditions. This was attributed to the higher C:N ratio of lupine field sowings caused by a restricted field germination rate and by the short incorporation time-frame of optimum seedling decomposability. An expanded seedling growing period of about 6 weeks resulted in a significantly lower net N mineralization compared to coarse meal, which was shown to be caused by a rapidly rising seedling C:N ratio and by the uptake of soil mineral N by the seedlings.

Results show conclusively that coarsely milled lupine seeds as well as seedlings being incorporated in early stages represent a viable N source for organic field vegetable production. Furthermore, it is indicated that the net N benefit from easily decomposable organic amendments can be effectively predicted by means of pot experiments.

2.1 Introduction

Since organic vegetable farms are mainly stockless, there is a lacking availability of manure as nitrogen (N) source. Legumes as N_2 fixing crops have the potential to contribute significant amounts of N to the following vegetable crop and thus play a major role in organic vegetable crop rotations. However, all traditional legume systems are characterized by the fact that neither the amount of N_2 fixed nor the time course of N mineralization can be influenced satisfactorily, making it hard to match the N demand of the following vegetable crop. The use of grain legume seeds of e.g. faba bean, lupine or pea as N fertilizer, which can be produced by the farmers themselves and which can be applied in well-controlled amounts flexibly in time and space, may overcome this problem. Coarsely milled legume seeds have been mentioned as valuable N fertilizer for organic horticulture. However, net N mineralization was rarely found to exceed 50% of total N in seed material (Schmitz and Fischer 2003; Stadler et al. 2006) even within incubation periods exceeding 60 days (Müller and von Fragstein und Niemsdorff 2006a). Reviewing comparative studies on animal- and plant-derived N fertilizers, Laber (2003) summarized that grain legume seeds generally showed a lower N release than animal-derived fertilizers or castor meal, a by-product of castor oil production.

Nitrogen mineralization of, in particular, tropical legume biomass was shown to be related to total lignin (L) and polyphenol (P) concentrations, L:N, P:N and (L+P):N ratios (Baijukya et al. 2006; Dinesh et al. 2001; Fox et al. 1990; Ibewiro et al. 2000; Oglesby and Fownes 1992) as well as to the protein binding capacity of polyphenols (Cadisch et al. 1998; Handayanto et al. 1997; Mafongoya et al. 1998). The concentration of polyphenols in legume seeds varies with species and quantitative as well as qualitative changes in the phenolic composition take place during seed germination (Lopez-Amoros et al. 2006). An increase in the total phenolic compounds content by 53% was reported by Fernandez-Orozco et al. (2006) in narrow-leaved lupines after 9 days of germination.

In numerous studies, the C:N ratio or the total N concentration (N_t) of incorporated crop residues have been found to explain most of the variation in net N mineralization (e.g. Trinsoutrot et al. 2000; Vigil and Kissel 1991). Depending on

the investigated plant materials and the range of their C:N ratios, an either linear (Chaves et al. 2004; Stadler et al. 2006) or curvilinear (Frankenberger and Abdelmagid 1985; Seneviratne 2000) decrease in net N mineralization with increasing C:N ratio was detected.

As an alternative to the seed application as coarse meal (CM), sowings at a high plant density (DS) can be established and incorporated after a short period. During seed germination storage compounds are broken down and used as energy source via respiration processes, resulting in the release of considerable amounts of CO_2 and as a consequence in a loss of total solids. Dagnia et al. (1992) found an apparent increase in protein content of 14.7% after 6 days of germination, which was attributed to the utilization of fats and carbohydrates by developing lupine seedlings. These findings were supported by Ghavidel and Prakash (2007) who calculated increases in seed protein content of four different legumes ranging from 6.1 to 9.7% within the first 36 hours of germination. The associated increase in N_t concentration and decrease in C:N ratio can be expected to enhance net N mineralization, if seedlings are incorporated in the soil well-timed.

It was hypothesized that for grain legume seeds and seedlings the C:N ratio is the predominant determinant of net N mineralization and that fertilizer utilization of seed N can be significantly improved by sowing compared to application as coarse seed meal.

2.2 Materials and methods

2.2.1 Pot experiment

A pot experiment was conducted consisting of a plant development and an incubation phase. Seeds of the low alkaloid narrow-leaved lupine (*Lupinus angustifolius* L.) cultivar ‘Boruta’ (5.1% N_t, C:N 8.8) were sown in pots filled with 2.74 kg of dry soil and a surface area of 1.77 dm^2 at densities of 24.4 seeds dm^{-2} corresponding to 173 kg N ha^{-1} or 111 mg N kg^{-1} of dry soil. Height of pots was 19 cm. The substrate was derived from the top layer (0-30 cm) of a Hapludalf soil

derived from loess (see field experiment). The initial soil mineral N (SMN) content was 21 mg N kg^{-1} of dry soil.

Germination and seedling development took place at 15°C and light intervals of 12 hours with light intensities of 16.2 W m^{-2} (PAR). Seedlings were cut into pieces of approximately 1.5 cm and mixed with the respective soils they had grown in before 8, 13, 18, 22, and 27 days after sowing. The growth stages attained ranged from the cotyledon stage eight days after sowing (DS-8) to the six-leaves stage 27 days after sowing (DS-27). A coarse seed meal treatment and an unamended control were established as references.

After incorporation, soils were incubated at 10 and 20°C and kept at 70% water holding capacity. Soil mineral N was determined 0, 15, 38, and 65 days after incorporation. Each treatment included five replicates per sampling.

2.2.2 Field experiment

Nitrogen mineralization of lupine coarse meal and seedlings under field conditions was studied in 2005 and 2006 using white cabbage (*Brassica oleracea* L. convar. *capitata* var. *capitata* f. *alba* cv. 'Impuls') as test crop. The experiments were located on organically managed fields at the university's experimental station in Ruthe, situated 15 km south of Hannover (52°14' N, 9°48' E). The soil was a Typic Hapludalf (FAO classification: Haplic Luvisol) developed from Weichselian loess deposits (>1.2 m) over Quaternary sand and gravel with approximately 10% sand, 80% silt and 10% clay. Soil organic matter content in the plough layer of 30 cm depth amounted to 1.4%.

Plots were arranged in a split-plot design with three replicates identically in each year. Main treatments consisted of four combinations of preceding crop and lupine seed type used for fertilization (Table 2.1). Precrops included two narrow-leaved lupine varieties of either low (cv. 'Boruta') or high alkaloid type (cv. 'Azuro') and spring wheat (*Triticum aestivum* L. cv. 'Thasos'). Lupine seeds used for fertilization were derived from crops in 2004 and showed seed C:N ratios of 8.5 (5.3% N_t) and 8.7 (5.1% N_t) for 'Boruta' and 'Azuro', respectively.

Table 2.1 Main treatments of the field experiment

Treatment	Preceding crop (2004 and 2005)	Lupine seed material for fertilization
I	*L. angustifolius* L. cv. 'Boruta'	*L. angustifolius* L. cv. 'Boruta'
II	*L. angustifolius* L. cv. 'Azuro'	*L. angustifolius* L. cv. 'Azuro'
III	*Triticum aestivum* L. cv. 'Thasos' (spring wheat)	*L. angustifolius* L. cv. 'Boruta'
IV	*Triticum aestivum* L. cv. 'Thasos' (spring wheat)	*L. angustifolius* L. cv. 'Azuro'

Three seed application treatments and an unamended control were assigned randomly as subplots to the main units. Lupine seed material was applied in amounts of 3.8 t ha^{-1} corresponding to 180 kg N ha^{-1} as dense sowing with either short or long developmental period until incorporation and as coarse meal. The dense sowing treatment with short developmental time (DS-S) was given 13 and 12 days to germinate and grow in 2005 and 2006, respectively, whereas the long time dense sowing treatment (DS-L) was incorporated 42 and 37 days after sowing in the two years. In 2005, seeds were broadcast with a manure spreader and a rotary harrow was used to incorporate seeds shallowly. Since germination rates were low in 2005 due to insufficient incorporation, seeds were drill-sown with a mechanical hand held device in 2006. Lupine coarse meal was applied by hand in both years. Organic matter of all treatments was incorporated with a rotary tiller on June 3 and June 1 in 2005 and 2006, respectively. Cabbage was transplanted five days after organic matter incorporation and harvested in mid October in both years.

Soil samples were collected to a depth of 120 cm in four layers of 30 cm each. Lupine seedlings were sampled including tap roots. In 2005, considerable amounts of weed biomass were removed from the plots by hand weeding. Weed samples were taken shortly prior to weeding to record the amount of N removed with weeds.

Daily average air temperature at two meter above-ground was obtained from a weather station located on the experimental site and was used to calculate temperature sums for lupine coarse meal and seedling mineralization.

2.2.3 Chemical analysis

Soil mineral N was extracted by addition of 0.1 N KCl solution. The extract was analyzed for NO_3^- and NH_4^+ using an UV spectrometer (Lambda 2S, PerkinElmer Inc., Waltham, MA, USA). Plant samples were dried at 70°C until weight constancy. Total C (C_t) and N (N_t) of lupine seeds and seedlings were analyzed by the Dumas method using an elemental analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany). Determination of total N in cabbage and weed samples was carried out by the micro-Kjeldahl procedure including nitrate reduction to ammonia.

2.2.4 Data analysis and statistics

Net N mineralization was calculated as difference between the amounts of N mineralized from unamended and amended soils and was expressed as percentage of total N applied with lupine seeds. The time course of net N mineralization was described by the single first-order equation

$$N_{net}(t) = (N_{tot\,max}(1 - e^{-kt})) - N_{upt}, \qquad [2.1]$$

where $N_{net}(t)$ is the net N mineralization at thermal time t as percentage of total N applied with seeds and k is the mineralization rate constant ($[°Cd]^{-1}$). Degree days were calculated by assuming 0°C as base temperature for organic matter decomposition. To allow for negative net N mineralization rates at incorporation date (t=0) caused by the growing lupine seedlings, N_{upt} represents the net N uptake by the seedlings until incorporation expressed as percentage of applied seed N. Negative values for N_{upt} indicate an N release from decomposing seeds prior to incorporation, particularly through decomposition of non-germinated seeds. As the total maximum net N mineralization $N_{tot\,max}$ represents the sum of the absolute values of $N_{net}(t=0)$ and $N_{net}(t\rightarrow\infty)$, the maximum net N mineralization $N_{net\,max}$ can be calculated as:

$$N_{net\,max} = N_{tot\,max} - N_{upt} \qquad [2.2]$$

Thermal time courses of net N mineralization under field conditions showed peaks at the first sampling dates in both years and in all treatments prior to level off until the end of the experiment. To quantify $N_{net\ max}$ according to the above described approach, mineralization peaks were initially excluded when fitting the single first-order kinetics model to the mineralization data. Due to exclusion of the first sampling date, the mineralization rate constant k could not be reasonably estimated by means of the remaining data points. Thus, k was allowed to range between the lowest and highest mineralization rate constant determined in the pot experiment only. The divergence between the first-order kinetics and the actually determined net N mineralization at the first sampling date was used as a measurement for the intensity of this short-term N dynamics and was termed N_{peak} (kg N ha^{-1}).

Seed coats were not recovered when taking seedling samples at incorporation date. This would have falsified comparisons of C:N ratio and N_t concentration between coarse meal and seedling biomass. Additionally, germination rates under field conditions were below 100%, particularly in 2005. Thus, sampled seedlings did not represent the total lupine biomass, necessitating adjustments of C:N ratio and N_t concentration. The adjusted N_t concentration (%) of lupine sowings was calculated as

$$N_t\ (\text{adjusted}) = \frac{N_{sdl} + N_{sc} + N_{ngs}}{DM_{sdl} + DM_{sc} + DM_{ngs}} \times 100\,, \qquad [2.3]$$

where N_{sdl}, N_{sc} and N_{ngs} (g m^{-2}) are the amounts of N in seedling and in seed coat biomass of the portion of germinated seeds and in non-germinated seeds, respectively. Dry matter amounts of the respective fractions are represented by DM_{sdl}, DM_{sc} and DM_{ngs} (g m^{-2}). Whereas DM_{sdl} was determined by sampling, dry matter of seed coat biomass of germinated seeds DM_{sc} was quantified as

$$DM_{sc} = DM_s \times \frac{SCP}{100} \times \frac{GR}{100}\,, \qquad [2.4]$$

where DM_s (g m^{-2}) is the total dry matter of applied seeds, SCP is the seed coat proportion of total seed dry matter, which was found to be 22.9%, and GR is the germination rate as percentage of the total number of seeds applied. The dry matter amount of non-germinated seeds (DM_{ngs}) was calculated as:

$$DM_{ngs} = DM_s \times \frac{100 - GR}{100} \quad [2.5]$$

Nitrogen amounts N_{sdl}, N_{sc} and N_{ngs} were derived by multiplying the respective dry matter amount by the corresponding N_t concentration. The N_t concentration of seedling biomass was determined within the sampling procedure for each plot separately, while the N_t concentration of lupine seed coats was quantified exemplarily in the pot experiment to be 0.39% (44.9% C_t). For the fraction of non-germinated seeds the N_t concentration of the total lupine seed as derived from the chemical analysis of lupine coarse meal was used. The adjustment of the C_t concentration was conducted in an analogous way and the adjusted C:N ratio was consequently derived by the adjusted C_t and N_t concentrations.

Differences between treatment means were analyzed by using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) was applied to the pot experimental data using the GLM procedure. Each year of the field experiment was analyzed separately by performing a two-way ANOVA accounting for the split-plot design using the MIXED procedure. Since neither main treatment effects nor factor interactions were observed, only mean values of main treatments are shown as results. If treatments were significantly different ($p \leq 0.05$), treatment means of pot and field experiment were separated by a Tukey and Tukey-Kramer HSD test at $p \leq 0.05$, respectively, if no other p -value is given. Percentage data were arcsin-transformed prior to analysis as far as data sets did not include negative values or values greater than 100% making transformation impossible. F-tests for parallelism (Rasch and Verdooren 2004) at $p \leq 0.05$ were carried out to compare dependencies of $N_{net\,max}$ on C:N ratio and N_t concentration between pot and field experiment by analysing differences in slopes and y-intercepts of the regression lines. Fitting of the single first-order kinetics model

and linear regressions were performed using SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany).

2.3 Results

2.3.1 Plant development

In the pot experiment between 77.3 and 93.8% of total C applied with seeds was recovered in lupine seedlings and seed coats (Fig. 2.1a). The lower value was recorded after 13 days of seedling development, indicating that 22.7% of total seed C was respired at this time. Net N uptake from soil started 10 days after sowing and increased linearly by 0.92 mg per kg of dry soil and day corresponding to 1.4 kg N ha^{-1} day^{-1} (referring to the pot soil surface) until the end of the experiment (Fig. 2.1b). The resultant C:N ratio reached its minimum of 6.2 after 13 days, which coincides with the lowest C_t content in seedlings (Fig. 2.1c). From day 13 on, seedling C:N ratio began to rise but stayed below the initial seed C:N ratio of 8.8 until the end of the experiment.

As in the pot experiment, the lowest C:N ratio under field conditions was recorded for the short lupine sowings (DS-S), which were incorporated after 13 and 12 days from sowing in 2005 and 2006, respectively (Table 2.2). During the additional growing time of 29 and 25 days of the long lupine sowings (DS-L), the C:N ratio increased and exceeded the mean seed C:N ratio of the two lupine varieties of 8.6 in both years. Since germination of lupine sowings was poor in 2005, the net amount of N recovered in seedling biomass ($N_{rec\ seedl}$) was low leading to a smaller differentiation in adjusted C:N ratio between treatments than in 2006. Although $N_{rec\ seedl}$ increased significantly with increasing seedling growing time in 2005, the sum of recovered mineral N in soil and in seedlings ($N_{rec\ sum}$) did not differ between the two sowing treatments. By contrast, in 2006 $N_{rec\ sum}$ was significantly lower for DS-L compared with the DS-S treatment, which was due to the additionally lower $N_{rec\ seedl}$ for DS-L.

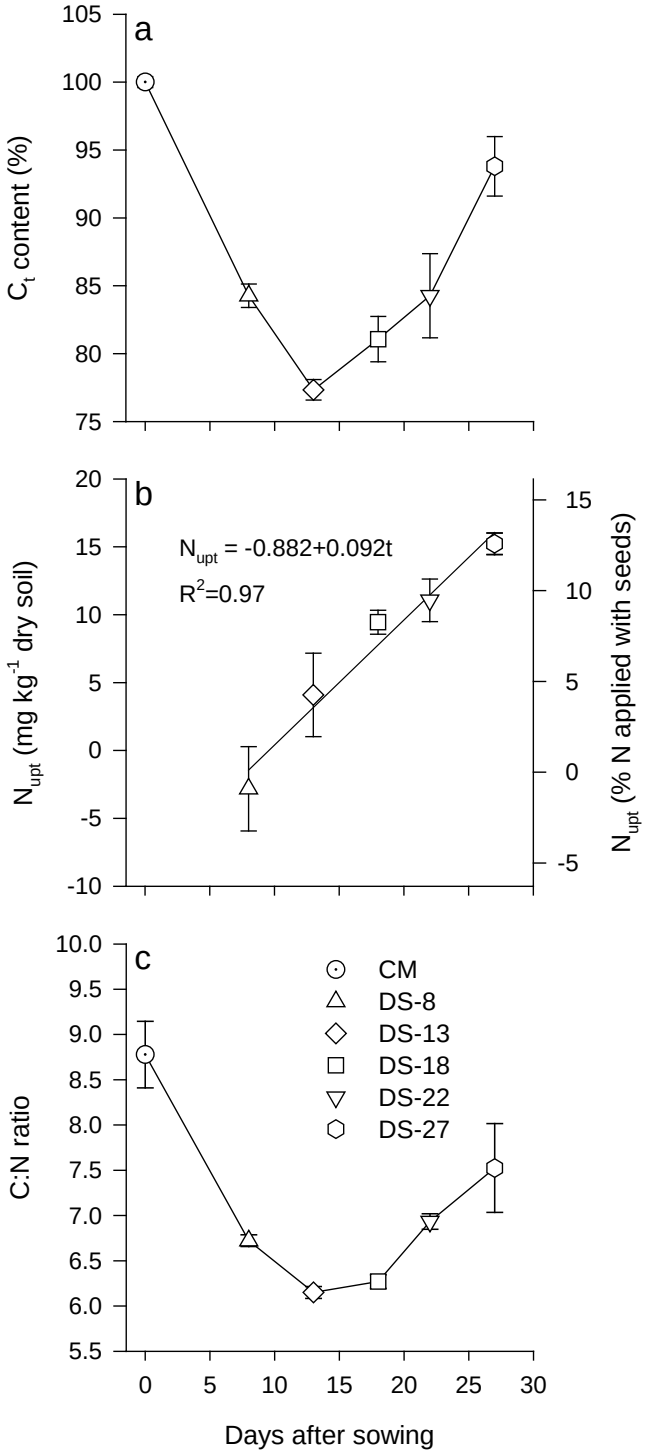

Fig. 2.1 Total C (C_t) content of lupine seedling and seed coat biomass as percentage of total C applied with seeds (a), net mineral N uptake (N_{upt}) (b) and C:N ratio (c) during lupine seed germination and early seedling development in the pot experiment. Lupine coarse meal (CM) corresponds to non-germinated seeds at day of sowing followed by sowing treatments with 8 (DS-8) to 27 days (DS-27) of seedling development. Error bars indicate standard deviations and are hidden by the symbol if not shown.

Table 2.2 Seedling dry matter, C:N ratio and N_t concentration of lupine sowings, net amounts of N recovered in seedlings ($N_{rec\ seedl}$) as soil mineral N ($N_{rec\ soil}$), and as sum of both ($N_{rec\ sum}$) expressed as percentage of total N applied with seeds for the short (DS-S) and long lupine dense sowing (DS-L) at incorporation date. Means of four main treatments (Table 2.1) and three replications.

	2005			2006		
	DS-S	DS-L	p	DS-S	DS-L	p
DM (g m^{-2})	82.1	270.1	<0.0001	208.8	357.9	<0.0001
C:N^z	8.1	9.5	<0.0001	7.0	10.9	<0.0001
$N_t{}^z$ (%)	5.3	4.7	<0.0001	5.6	4.0	<0.0001
$N_{rec\ seedl}$ (%)	33.4	51.9	<0.0001	84.4	68.7	0.0026
$N_{rec\ soil}$ (%)	25.8	3.6	0.0247	4.2	-21.4	0.0028
$N_{rec\ sum}$ (%)	59.2	55.5	NS	88.6	47.3	0.0009

NS = non-significant; n=3.

^{z}Adjusted for non-recovered seed coat biomass and non-germinated seeds.

2.3.2 Nitrogen mineralization

Nitrogen uptake by lupine seedlings in the pot experiment resulted in negative values for net N mineralization at incorporation date with exception of the youngest seedlings of 8 days of age (DS-8) (Fig. 2.2). Nitrogen release of all lupine materials started rapidly after incorporation without any lag phase. After an incubation period of 1300°Cd, net N mineralization of all sowing treatments was significantly higher than of CM. Within sowing treatments, net N mineralization was found to be in the reverse order of seedling growing time, being highest for seedlings incorporated already after 8 days. Since the first-order kinetics model fitted highly significantly (p<0.01) to the mineralization data, the same ranking was found for $N_{net\ max}$ (Table 2.3). However, values for $N_{net\ max}$ were between 3 and 12% lower than the actually quantified net N mineralization after a thermal incubation time of 1300°Cd. The mineralization rate constant k did not vary markedly between treatments except for DS-8 where k was noticeably lower. To explain differences in net N mineralization between treatments, $N_{net\ max}$ was related to both C:N ratio and N_t concentration, yielding the following equations:

$$N_{net\ max} = 134.9 - 8.8\ C{:}N \qquad R^2=0.87 \qquad [2.6]$$

$$N_{net\ max} = -12.3 + 14.5\ N_t \qquad R^2=0.82 \qquad [2.7]$$

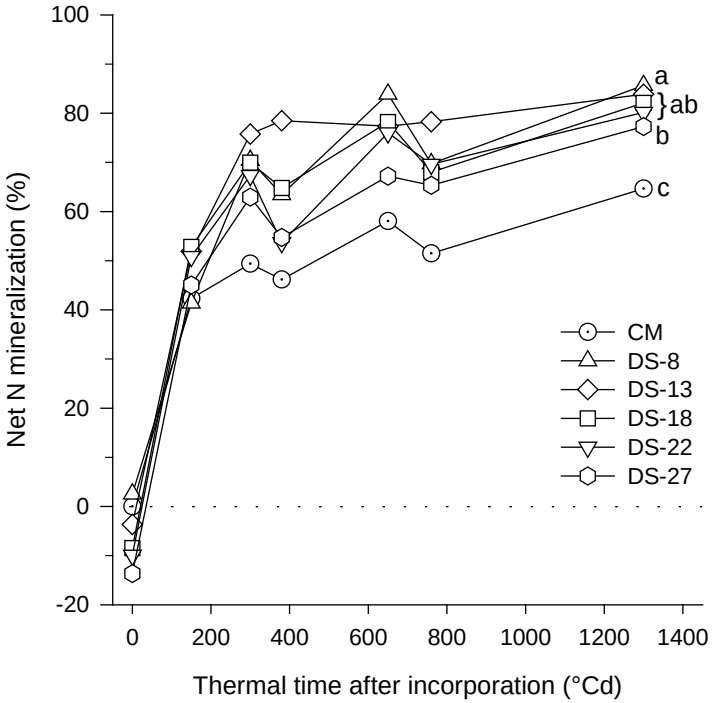

Fig. 2.2 Net N mineralization of lupine seedlings incorporated after 8 (DS-8) to 27 days (DS-27) from sowing and lupine coarse meal (CM) at 10 and 20°C in the pot experiment. Different letters indicate significant differences after 1300°Cd (Tukey HSD, $p \leq 0.05$, n=5).

At incorporation date of field established lupine sowings, net N mineralization varied between -21 and 26%, depending on germination rate and seedling growing time (Fig. 2.3). In the CM treatment in both years and DS-S in 2006 net N mineralization exceeded the quantity of lupine seed N added within the first 640 and 545°Cd after incorporation in 2005 and 2006, respectively. After peaking, however, net N mineralization decreased to proportions between 52 and 64% during the following 597 and 654°Cd and remained in the range of 37 to 72% until cabbage harvest. Even though net N mineralization of DS-L in both years and of

Table 2.3 Mineralization parameters as derived from the first-order kinetics model fitted to the pot experimental data, resulting maximum net N mineralization ($N_{net\ max}$) and thermal time at which 95% of $N_{net\ max}$ was achieved (t_{95}). Parenthesized values represent standard deviations.

Treatment	k ([°Cd]$^{-1}$)	$N_{tot\ max}$ (%)	N_{upt} (%)	R^2	$N_{net\ max}$ (%)	t_{95} (°Cd)
CM	0.0074 (0.0024)	55.8 (7.1)	-0.8 (6.3)	0.94	56.6	403
DS-8	0.0049 (0.0013)	79.1 (8.8)	-2.3 (7.5)	0.95	81.4	605
DS-13	0.0077 (0.0009)	85.2 (3.7)	4.0 (3.3)	0.99	81.1	395
DS-18	0.0084 (0.0019)	83.2 (7.1)	8.2 (6.3)	0.97	75.0	369
DS-22	0.0076 (0.0024)	81.9 (10.0)	9.0 (8.9)	0.94	72.8	410
DS-27	0.0076 (0.0018)	81.8 (7.7)	13.1 (6.8)	0.97	68.7	417

DS-S in 2005 did not exceed 100% at the first sampling, a similar mineralization pattern including a peak during the early stage of decomposition was detectable as well. Net N mineralization achieved by CM at cabbage harvest was not significantly increased by any sowing treatment but was even significantly higher than DS-L in both years. The actually quantified mineralization rates at this time were slightly underestimated by an overall mean of 6% by $N_{net\ max}$ deriving from the first-order kinetics. Linear relationships between $N_{net\ max}$ and C:N ratio or N_t concentration for the field experiment resulted in the following dependencies:

$$N_{net\ max} = 132.4 - 9.8\ C{:}N \qquad R^2=0.52 \qquad [2.8]$$

$$N_{net\ max} = -78.6 + 25.0\ N_t \qquad R^2=0.59 \qquad [2.9]$$

In relation to its C:N ratio or N_t concentration, $N_{net\ max}$ of CM in 2005 was considerably underestimated by the linear regressions and clearly lowered the coefficients of determination.

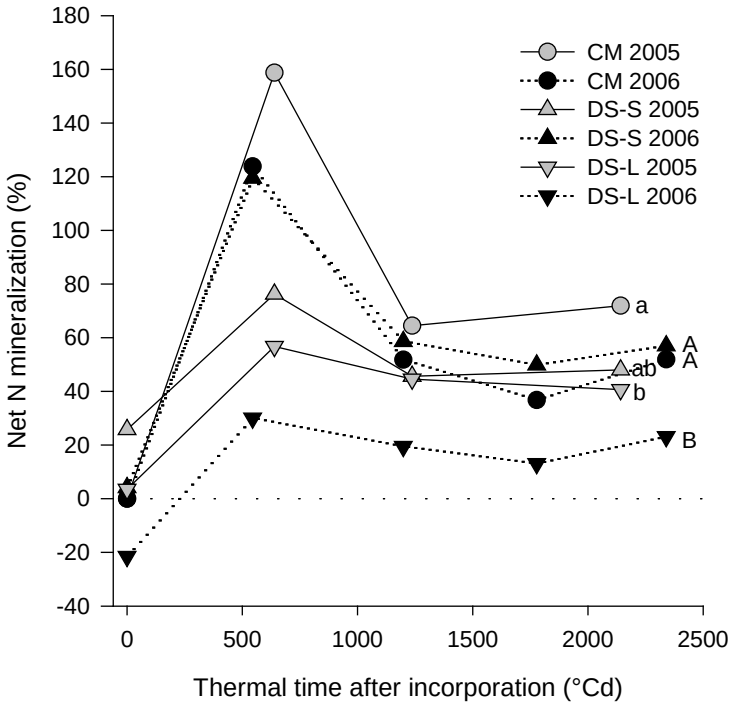

Fig. 2.3 Net N mineralization of lupine seedlings with either short (DS-S) or long developmental time (DS-L) until incorporation and of lupine coarse meal (CM) in the field experiment. Means of four main treatments (Table 2-1) and three replications. Different letters indicate significant differences at cabbage harvest in 2005 (small letters) and 2006 (capital letters) at $p \leq 0.05$ (Tukey - Kramer HSD).

The divergence between the first-order kinetics and the actually determined net N mineralization at peaking (N_{peak}) was used as a measure for peaking intensity. Values for N_{peak} were highest for CM and lowest for DS-L in both years (Table 2.4). Even if DS-S caused N_{peak} values ranging between CM and DS-L in both years, N_{peak} induced by DS-S was noticeably higher in 2006 than 2005.

Table 2.4 Net uptake of soil mineral N by lupine seedlings (N_{upt}) and maximum net N mineralization ($N_{net\ max}$) as derived from the first-order kinetics model fitted to the field experimental data and difference between the first-order kinetics and the actually measured net N mineralization at peaking (N_{peak}) for the coarse meal treatment (CM) and the short (DS-S) and long dense sowing (DS-L). Means of four main treatments (Table 2.1) and three replications. Parenthesized values represent standard deviations of main treatments. Different letters indicate significant differences in 2005 (lower case letters) and 2006 (capital letters) at α 0.05 (Tukey - Kramer HSD).

	2005			2006		
	CM	DS-S	DS-L	CM	DS-S	DS-L
N_{upt} (%)	0.0 (<0.1)	-25.8 (5.2)	-3.6 (18.6)	0.0 (<0.1)	-4.2 (7.6)	21.5 (3.6)
$N_{net\ max}$ (%)	68.3 (7.6)	46.8 (6.8)	42.7 (6.1)	46.9 (7.7)	55.2 (7.1)	18.6 (13.5)
R^2	0.87 - >0.99	0.73 - >0.99	0.90 - 0.98	0.55 - >0.99	0.88 - 0.98	0.83 - 0.92
N_{peak} (kg ha^{-1})	166.7a	54.2b	26.7b	141.9A	118.5A	23.4B

2.3.3 Comparison of pot and field experiments

Dependencies of $N_{net\ max}$ on C:N ratio (equations 2.6 and 2.8) and N_t concentration (equations 2.7 and 2.9) were compared to test for differences between pot and field experiment. Neither differences in intercepts nor in slopes between regression lines were detectable, irrespective of using C:N ratio or N_t concentration as explanatory variable. Thus, data sets of pot and field experiment were pooled. The resulting relationships between $N_{net\ max}$ and C:N ratio or N_t concentration are shown in Figure 2-4. The critical C:N ratio, i.e. the C:N ratio at the break-even point between net N mineralization and immobilization, was found to be 13.1.

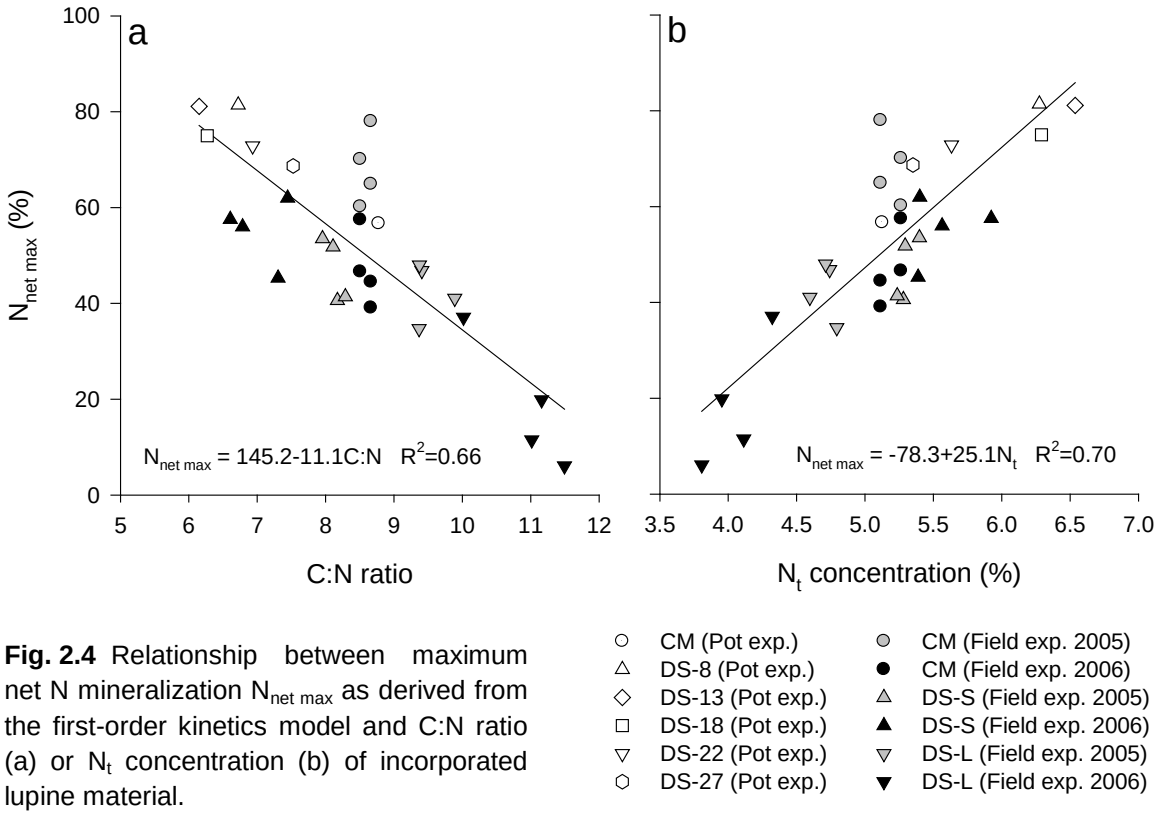

Fig. 2.4 Relationship between maximum net N mineralization $N_{net\ max}$ as derived from the first-order kinetics model and C:N ratio (a) or N_t concentration (b) of incorporated lupine material.

2.4 Discussion

Pot experiments carried out under laboratory conditions are an effective tool for the detailed analysis of plant and soil processes, since disturbing influences as occurring in the field can be excluded. In the presented laboratory study, germination of lupine seeds was accompanied by a considerable net release of CO_2-C amounting up to 23% of initial total C in seeds and the associated C:N ratio reached its minimum of 6.2 after 13 days from sowing. Since net N uptake by seedlings did not start before 10 days after sowing, the decrease in C:N ratio can rather be attributed to the concentration of seed N due to a respiration based mass loss than to uptake of soil mineral N. The C:N ratio of all observed lupine materials was low enough for net N mineralization to start immediately after incorporation without any initial immobilization or lag phase, which is a pre-condition for the use of exponential models when describing N mineralization as a function of time (Chaves et al. 2004). The mineralization rate constant k as derived from the single first-order model did not vary markedly between

treatments except for the youngest seedlings of 8 days where k was apparently lower. This was due to an N release of decomposing seeds prior to incorporation, which is indicated by the negative value for net N uptake by seedlings. By contrast, $N_{net\ max}$ was significantly related to both the altered C:N ratio and N_t concentration, corroborating our hypothesis of enhancing N mineralization by an altered seed chemical composition due to germination.

However, pot experiments represent artificial systems and may only partly reflect *in situ* conditions (Passioura 2006). The transferability of mineralization data obtained under laboratory conditions to field realities might therefore be viewed critically (Jarvis et al. 1996; Rasmussen et al. 1998). Dense sowings in the field resulted in lower germination rates compared to the pot experiment raising the question about the fate of N in non-germinated seeds. In 2005, net amounts of N recovered in seedlings increased with increasing seedling growing time, whereas the sum of seed N recovered in soil and seedlings did not differ between the two sowing treatments. Thus, the amount of N released by decaying seeds within the first 13 days was nearly completely taken up by seedlings during the additional 29 days growing time of the DS-L treatment. The depletion of SMN during the additional growing periods of 29 and 25 days in 2005 and 2006, respectively, corresponded to 1.4 and 1.8 kg N ha^{-1} day^{-1}, which is in accordance with the N uptake of 1.4 kg ha^{-1} day^{-1} determined in the pot experiment. However, in 2006, net amounts of N recovered in seedlings were found to be lower for the DS-L treatment and did not account for depletion in SMN. This was probably due to a dying back of seedlings caused by the high initial plant density.

The most striking difference between pot and field experiment consisted in the occurrence of initial net N mineralization peaks in the field. Mineralization rates exceeding 100% of added seed N indicate that decomposition of lupine materials triggered an additional mineralization of native soil organic N, a phenomenon that is commonly known as positive priming effect. Priming effects had been defined as strong short-term changes in SOM turnover caused by comparatively moderate treatments of the soil and their size was shown to increase with the amount of the added organic substance (Kuzyakov et al. 2000). The latter is supported by the high peaking intensity observed for the coarse meal treatment compared to the

dense sowings where the restricted seed germination lead to a reduced lupine biomass at incorporation date. A long development period of lupine sowings prior to incorporation caused only small peaking intensities indicating that the quality of incorporated plant material was another major determinant of the priming effect. Positive priming effects after soil incorporation of easily available substrate are often supposed to result from an accelerated degradation of SOM. Complex interactions between soil organisms (Fontaine et al. 2004; Fontaine et al. 2003) as well as an increased abundance of extracellular enzymes deriving from the activated soil microbial biomass (SMB) (Asmar et al. 1994) have been identified as driving mechanisms. By contrast, other studies found that additionally released CO_2 after the addition of organic substrates derived directly from SMB (Dalenberg and Jager 1981; 1989; Wu et al. 1993). Net priming effects inducted by coarse legume seed meals have been observed in incubation trails (Müller and von Fragstein und Niemsdorff 2006a) and field experiments (Müller and von Fragstein und Niemsdorff 2006b). The calculated recovery applied coarse meal N in K_2SO_4-extractable SOM, SMB and as SMN amounted up to 139%. In contrast, net priming effects observed in the present study were exclusively based on differences in SMN and occurred as peaks, which implies a substantial loss of SMN after peaking. Since no accumulation of potentially leachable N was detected in lower soil layers at any sampling date and denitrification is expected to be restricted by the prevalent soil water content (Aulakh et al. 1991; Del Prado et al. 2006; Liu et al. 2007), the decline in SMN can be ascribed to immobilization. A similar mineralization pattern with a peak exceeding the amount of plant residue N added was found by Bending et al. (1998) in an incubation study. They suggested that immobilization into SOM after passing SMB contributed to the loss of SMN.

Despite ignoring priming peaks when calculating $N_{net\ max}$, the dependencies of $N_{net\ max}$ on C:N ratio or N_t concentration were not found to differ significantly between pot and field experiment. Thus, initial priming peaks can be assumed to have had a minor effect on medium-term N mineralization of incorporated lupine materials. However, under field conditions net N mineralization was not significantly increased by sowing compared to the application as coarse meal. This discrepancy to the pot experiment is attributed to the higher C:N ratio of

lupine sowings in the field caused by the restricted germination rate and the short incorporation time-frame of optimum seedling decomposability. In addition, a positive influence of the priming effect on $N_{net\ max}$ could not be ruled out completely and might explain the tendency of the comparatively high value for $N_{net\ max}$ of the CM treatment in 2005.

Maximum net N mineralization was shown to decrease strongly with an increasing seedling C:N ratio resulting in a comparatively low critical C:N ratio of 13.1 (cp. Chaves et al. 2004; Odhiambo and Bomke 2000; Seneviratne 2000; Stadler et al. 2006). Since the C:N ratio did not account for the reduction in SMN due to seedling growth, the critical C:N ratio can be assumed to be influenced by the N uptake of seedlings. The highest net N uptakes were recorded for the DS-L treatments under field conditions in 2006 and thus might have contributed to the low critical C:N ratio.

2.5 Conclusions

Both C:N ratio and N_t concentration of coarsely milled lupine seeds and seedlings differing in age provide similarly good indicators for their net N mineralization. The initial decrease in C:N ratio during seed germination is of limited practical relevance, as the correspondingly early stage of seedlings does not allow for their satisfactory soil incorporation. Thus, if lupine seedlings are incorporated as early as practically possible, the resulting net N mineralization is comparable to that from lupine coarse meal. A delayed incorporation, e.g. due to inappropriate weather and soil conditions or seasonal work peaks, bears the risk of a reduced net N mineralization caused by the rapidly rising C:N ratio and additionally by the uptake of SMN by seedlings.

Furthermore, it is concluded that the net N benefit of N-rich organic amendments within the year of their application can be successfully predicted by means of incubation experiments. Thus, although organic N fertilizers have been largely investigated by incubation, available data on their net N mineralization can be considered as valuable for practical application. However, ‘short-term turbulences’ in the soil N dynamics, which can be induced by field incorporation of easily

decomposable organic materials, are not reflected by using heavily disturbed soils as is common practice in laboratory experiments.

3 Nitrogen use efficiency of organically fertilized white cabbage and residual effects on subsequent beetroot

Abstract

The generally low N availability in organic vegetable production requires an efficient use of limited N sources to assure crop yield and quality. Seeds of grain legumes are comparatively rich in N and may therefore be utilized as N fertilizer. In 2005 and 2006, field experiments on a loess-derived Typic Hapludalf soil were carried out to investigate effects of soil-incorporated lupine seedlings of different ages and coarsely milled lupine seeds on the N use efficiency (NUE) of white cabbage and on individual NUE components. Subsequent to cabbage in 2005, a beetroot crop was established in 2006 to quantify residual effects of lupine amendments.

In general, cabbage NUE decreased with increasing N availability, which was due to an increasing concentration of total N in above-ground cabbage biomass. In contrast, N uptake efficiency was found to increase curvilinearly to an asymptotic maximum, while yield fresh matter formation index, i.e. the inverse of dry matter concentration in cabbage heads, increased linearly with available N. Dry matter concentration and harvest index were generally lower in 2005, when thermal time from transplanting to harvest was shorter than in 2006. In 2005, harvest index was considerably lower after incorporation of six weeks old lupine seedlings compared to seedlings of 13 days age or lupine coarse meal. The lower harvest index was associated with a hampered early cabbage growth.

Nitrogen supply for beetroot was increased by lupine amendments by on average 18% of lupine seed N applied in the previous year. This residual effect was attributed to incremental N in cabbage residues, while potentially late mineralizing lupine seed N did not contribute observably to beetroot N supply.

It is concluded that suboptimal crop establishment delays cabbage maturation, which can prevent the crop from reaching maximum harvest index within the given growth period. Ensuring optimal early growth can therefore be crucial for realizing the full yield potential and consequently for high NUE. The residual effect of organic N fertilizers depends largely on the re-utilization of incremental fertilizer N in cabbage residues by the succeeding crop and as a consequence additionally on net N mineralization of the organic fertilizer within the year of application.

3.1 Introduction

Grain legume seeds of e.g. faba bean, lupine or pea are currently discussed as nitrogen (N) fertilizer in organic vegetable production. They are comparatively rich in N and can be produced by farmers themselves and applied in well-controlled amounts flexibly in time and space. Such a cropping system would combine the possibility of utilizing legume N_2 fixation as local N source with the high flexibility and controllability of commercial organic fertilizers. As an alternative to their application as coarsely milled seeds (Müller and von Fragstein und Niemsdorff 2006a; 2006b; Stadler et al. 2006), sowings with a correspondingly high plant density can be established and incorporated after a short period. In a previous study, we investigated the release of mineral N from coarsely milled lupine seeds and lupine seedlings of different ages in field experiments with white cabbage (see Chapter 2). It was found that net N mineralization of lupine materials was negatively related to their C:N ratio, which increased with seedling age. However, differences between seed application methods in the efficiency with which mineralized lupine N contributed to cabbage yield formation as well as residual effects on a subsequent crop remain open questions.

In general, the efficiency of a cropping system in using N depends on both soil and plant related processes (Huggins and Pan 1993) and may be described by differently defined input to output ratios (Fageria and Baligar 2005; Good et al. 2004; Huggins and Pan 2003). On a crop level, N use efficiency (NUE) was defined by Moll et al. (1982) as fresh matter yield per unit of plant available N. Mathematically, NUE may be split into multiplicative sub efficiencies, which are related to major plant physiological processes. According to Moll et al. (1982), the two primary components of NUE are the efficiency of soil mineral N uptake (plant N per unit of plant available N) and the utilization efficiency with which plant N is converted into yield (fresh matter yield per unit of plant N). Under the same cropping and soil conditions, NUE typically decreases with increasing N supply (Fageria and Baligar 2005; Hirel and Lemaire 2005). As NUE decreases, yield incremental gains become smaller resulting in the typical curvilinear relationship between yield and N supply, which is commonly described by quadratic equations or variants of the Mitscherlich equation. Expected differences in cabbage NUE

between lupine treatments will therefore to a certain extent be due to differences in the amount of plant available N. On the other hand, field application of lupine seeds by sowing followed by the incorporation of seedlings can be considered as a short-term green manure crop, which might affect growth and yield formation of cabbage and consequently NUE independently of available N. If NUE is closely related to available N, the latter effect can be considered to be small. In turn, a weak relationship between NUE and available N would indicate non-N effects.

Therefore, one aim of the present study was to quantify effects of above described lupine amendments on NUE of white cabbage and to identify individual NUE components being responsible for differences in overall NUE.

We previously observed net N mineralization of lupine materials within the cabbage growth period to vary between 23 and 67%, depending on the method of lupine seed application. Similarly, net N mineralization of more commonly used organic N fertilizers like meal of horn or feather or de-oiled castor cake during crop growth or comparable incubation periods was reported to range principally between 30 and 70% (Cordovil et al. 2007; Cordovil et al. 2005; Hadas and Kautsky 1994; Hartz and Johnstone 2006; Müller and von Fragstein und Niemsdorff 2006a; 2006b; Stadler et al. 2006). In turn, a significant proportion of N added with organic N fertilizers in general may not contribute to the N supply for the fertilized vegetable crop but will either be available to a subsequent crop, lost by leaching or will contribute to the fraction of slowly decomposing soil organic matter. Gutser et al. (2005) stated that the residual effect of organic fertilizers increases with decreasing short-term net N mineralization (N effect in the year of application) and is especially important when short-term net N mineralization is below 70%. Müller and von Fragstein und Niemsdorff (2006b) investigated the fertilizer use of castor cake and coarsely milled lupine seeds in a field experiment. On average of both fertilizers and different application rates 19 and 42% of applied fertilizer N was recovered as soil mineral N and easily mineralizable organic N at harvest of white cabbage and radish, respectively.

Consequently, we hypothesized to find residual effects of agronomically relevant magnitude after soil incorporation of lupine seeds and seedlings. Furthermore, we expected residual effects to be the larger, the lower the net N mineralization in the year of application.

3.2 Materials and methods

3.2.1 Experimental setup and sampling

In 2005 and 2006, field experiments were carried out to investigate the use of lupine seeds as organic N fertilizer for white cabbage (*Brassica oleracea* L. convar. *capitata* var. *capitata* f. *alba* cv. 'Impuls'). A subsequent beetroot (*Beta vulgaris* L. var. *conditiva* cv. 'Detroit 2/Bolivar') crop was established in 2006 following cabbage grown in 2005. The experiments were located on organically managed fields at the university's experimental station in Ruthe, situated 15 km south of Hannover (52°14' N, 9°48' E). The soil was a Typic Hapludalf (FAO classification: Haplic Luvisol) developed from Weichselian loess deposits (>1.2 m) over Quaternary sand and gravel with approximately 10% sand, 80% silt and 10% clay. Soil organic matter content in the plough layer of 30 cm depth amounted to 1.4%.

Plots were arranged in a split-plot design with three replicates identically in each year. Main treatments consisted of four combinations of preceding crop and lupine seed type used for fertilization (Table 3.1). Precrops included two narrow-leaved lupine (*Lupinus angustifolius* L.) varieties of either low (cv. 'Boruta') or high alkaloid type (cv. 'Azuro') and spring wheat (*Triticum aestivum* L. cv. 'Thasos').

Table 3.1 Main treatments of the field experiment

Treatment	Preceding crop (2004 and 2005)	Lupine seed material for fertilization
I	*L. angustifolius* L. cv. 'Boruta'	*L. angustifolius* L. cv. 'Boruta'
II	*L. angustifolius* L. cv. 'Azuro'	*L. angustifolius* L. cv. 'Azuro'
III	*Triticum aestivum* L. cv. 'Thasos' (spring wheat)	*L. angustifolius* L. cv. 'Boruta'
IV	*Triticum aestivum* L. cv. 'Thasos' (spring wheat)	*L. angustifolius* L. cv. 'Azuro'

Lupine seeds used for fertilization were derived from precrops in 2004. Three seed application treatments and an unamended control were assigned randomly as subplots to the main units. In 2005 and 2006, spring soil samples prior to white cabbage crops were taken on April 4 and April 18, respectively. Lupine seed material was applied in amounts of 3.8 t ha^{-1} corresponding to 180 kg N ha^{-1} as dense sowing with either short or long developmental period until incorporation and as coarse meal. The dense sowing treatment with short developmental time (DS-S) was given 13 and 12 days to germinate and grow, whereas the long time dense sowing treatment (DS-L) was incorporated 42 and 37 days after sowing in 2005 and 2006, respectively. Organic matter of all treatments was incorporated with a rotary tiller in early June in both years. Cabbage was transplanted five days after organic matter incorporation at a density of 4.3 plants m^{-2}. The cabbage growth period from transplanting to harvest lasted 131 days in 2005 and 126 days in 2006. Assuming a base temperature of 10°C (Isenberg et al. 1975), the growth period corresponded to a temperature sum of 782 and 1027°Cd in 2005 and 2006, respectively. Cabbage above-ground biomass was sampled at harvest, separating cabbage head and harvest residues, as well as during crop growth. The first interim sampling was carried out 35 and 28 days after transplanting in 2005 and 2006, respectively. In 2005, cabbage was sprinkler irrigated twice in July, while in 2006 irrigation was performed immediately after transplanting and during early crop growth with a customized tractor mounted spray system. Cabbage harvest residues were incorporated by ploughing on November 8, 2005. Spring soil samples were taken on May 9, 2006. A subsequent beetroot crop was sown and harvested on June 27 and September 20, 2006, respectively. The realized plant density averaged 35 plants m^{-2}.

Soil samples were collected to a depth of 120 cm in four layers of 30 cm each. In 2005, considerable amounts of weed biomass were removed from cabbage plots by hand weeding at the end of July. Weed samples were taken shortly prior to weeding to record the amount of N removed with weeds.

3.2.2 Chemical analysis

Soil mineral N (SMN) was extracted by addition of 0.1 N KCl solution. The extract was analyzed for NO_3^- and NH_4^+ using an UV spectrometer (Lambda 2S, PerkinElmer Inc., Waltham, MA, USA). Plant samples were dried at 70°C until weight constancy and analyzed for total N (N_t) by the micro-Kjeldahl procedure including nitrate reduction to ammonia.

3.2.3 Data analysis and statistics

The total amount of available N (N_{av}) for white cabbage and beetroot was quantified by summing total above-ground plant N (N_p) and residual SMN present to a soil depth of 120 cm at harvest. Net N mineralization from applied lupine seed material was calculated as difference in N_{av} between amended soils and unamended control soils. The release of N from soil organic matter (SOM) during the growing periods of cabbage and beetroot was derived from unamended control soils and was computed as difference between SMN in spring before crop establishment and N_{av} at crop harvest. Over-winter net N mineralization was calculated as difference between residual SMN at cabbage harvest and spring SMN present to a soil depth of 120 cm.

To account for potential N losses during the winter leaching period, the soil water balance was computed using weather data recorded at the experimental station. The reference evapotranspiration ET_0 was obtained by the FAO Penman-Monteith equation (Allen et al. 1998) on a daily basis using maximum and minimum values of temperature and relative humidity, daily average wind speed and solar radiation as input parameters. The actual evapotranspiration ET_c was obtained by multiplying ET_0 by the crop specific coefficient K_c. To calculate the actual evaporation from bare soil after cabbage harvest, the dual crop coefficient approach was applied, in which K_c is split into the basal crop coefficient K_{cb} and the evaporation coefficient K_e (Allen et al. 1998). Since K_{cb} primarily represents the transpiration component, K_{cb} was set to zero by reason of the bare soil during winter. The evaporation coefficient K_e was computed on a daily basis depending on the total amount of water, which was able to evaporate from the soil surface layer of 10 cm (Allen et al. 1998). The amount of leachate draining to below

120 cm soil depth was derived by consideration of the initial soil water content (SWC) at cabbage harvest in 2005, the soil specific field capacity with respect to a soil depth of 120 cm and the computed soil water balance.

Nitrogen use efficiency of cabbage (NUE) was defined as fresh matter yield (Y_{fm}) per unit available N (N_{av}) and was subdivided according to Moll et al. (1982) into two main components: The efficiency of soil mineral N uptake (N_p/N_{av}) and the N utilization efficiency (Y_{fm}/N_p) with which N_p is converted into fresh matter yield (Y_{fm}) (Table 3.2):

$$Y_{fm}/N_{av} = (N_p/N_{av})(Y_{fm}/N_p) \quad [3.1]$$

Nitrogen utilization efficiency was further compartmentalized into N utilization efficiency for total dry matter production (P_{dm}/N_p), dry matter harvest index (Y_{dm}/P_{dm}) and yield fresh matter formation index (Y_{fm}/Y_{dm}) (Table 3.2). Therefore, NUE of white cabbage can be expressed as:

$$Y_{fm}/N_{av} = (N_p/N_{av})(P_{dm}/N_p)(Y_{dm}/P_{dm})(Y_{fm}/Y_{dm}) \quad [3.2]$$

Nitrogen utilization efficiency for total dry matter production represents the inverse of the N_t concentration in total plant dry matter and yield fresh matter formation index represents the inverse of the dry matter concentration in cabbage heads.

Multiplying N uptake efficiency by N utilization efficiency for total dry matter production results in the ratio of total above-ground plant dry matter to total available N (P_{dm}/N_{av}), which was termed N use efficiency for total dry matter production (Table 3.2):

$$P_{dm}/N_{av} = (N_p/N_{av})(P_{dm}/N_p) \quad [3.3]$$

Differences between treatment means were analyzed by using SAS 9.1 (SAS Institute Inc., Cary, NC, USA). Experimental years were analyzed separately by performing a two-way ANOVA. Additionally, a two-way ANCOVA with N_{av} as covariate was carried out to test for differences between lupine treatments on

NUE and its components independently of N_{av}. For both analyses, the MIXED procedure was used accounting for the split-plot design. Since main treatment effects were rarely significant and generally small and since no factor interactions were observed, only mean values of main treatments are shown as results. If treatments were significantly different ($p \leq 0.05$), treatment means were separated by Tukey-Kramer HSD test at $p \leq 0.05$. Regression analysis was performed using SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany). Regressions were considered significant if the overall model p-value was ≤ 0.05 and are only shown if significant.

Table 3.2 Definition of yield parameters and N efficiency terms

Y_{fm}	fresh matter yield	t ha^{-1}
Y_{dm}	dry matter yield	g m^{-2}
N_y	crop yield N	kg ha^{-1}
N_p	total above-ground plant N	kg ha^{-1}
P_{dm}	total above-ground plant dry matter	g m^{-2}
N_{av}	total available N (N supply)	kg ha^{-1}
Y_{fm}/N_{av}	N use efficiency (NUE)	kg kg^{-1}
P_{dm}/N_{av}	N use efficiency for total dry matter production	kg kg^{-1}
N_p/N_{av}	N uptake efficiency	kg kg^{-1}
Y_{fm}/N_p	N utilization efficiency	kg kg^{-1}
P_{dm}/N_p	N utilization efficiency for total dry matter production	kg kg^{-1}
Y_{dm}/P_{dm}	dry matter harvest index	kg kg^{-1}
N_y/N_p	N harvest index	kg kg^{-1}
Y_{fm}/Y_{dm}	yield fresh matter formation index	kg kg^{-1}

3.3 Results

3.3.1 Cabbage N supply and yield parameters

Depending on the method of lupine seed application, N_{av} for white cabbage was increased by the fertilizer treatments by 68 to 120 kg ha^{-1} in 2005 and 42 to 103 kg ha^{-1} in 2006 (Table 3.3). In 2006, indigenous soil N supply composed of SMN present in spring and N mineralized from SOM during cabbage growth period was 96 kg ha^{-1} higher compared to 2005. In 2005, on average 23 kg N ha^{-1}

were removed from the plots with weed biomass. The resulting N_{av} for cabbage ranged from 189 to 308 kg ha^{-1} in 2005 and 301 to 404 kg ha^{-1} in 2006.

Table 3.3 Amount of total available N (N_{av}) for white cabbage as composed of spring soil mineral N (SMN), net N mineralization from lupine seed material and soil organic matter (SOM) during cabbage growth period and weed N uptake for the unamended control (Ctrl), the coarse meal treatment (CM) and the short (DS-S) and long dense sowing (DS-L). Means of four main treatments (Table 3.1) and three replications. Different letters indicate significant differences between treatments in 2005 (lower case letters) and 2006 (capital letters) at $p \leq 0.05$ (Tukey - Kramer HSD).

	2005				2006			
	Ctrl	CM	DS-S	DS-L	Ctrl	CM	DS-S	DS-L
N_{av} (kg ha^{-1})	188.6c	308.3a	267.1ab	256.6b	301.2B	394.7A	403.8A	342.9B
Spring SMN (kg ha^{-1})	25.2				129.0			
Lupine seed net N mineralization (kg ha^{-1})	-	129.5a	86.4ab	73.3b	-	93.5A	102.6A	41.7B
SOM net N mineralization (kg ha^{-1})	179.5				172.1			
Weed N uptake (kg ha^{-1})	16.8a	26.5a	24.7a	22.1a	-	-	-	-

Compared to the unfertilized control, cabbage yield parameters were significantly higher when lupine seed material was applied as coarse meal or early incorporated dense sowing in both years, except for the amount of cabbage head dry matter in 2006 (Table 3.4). The same amount of lupine seeds applied as a late incorporated sowing had a significant positive effect only in 2005 on total above-ground plant N and on the amount of N in cabbage yield. The incremental amount of N in cabbage yield due to lupine amendments ranged from 25 to 52 kg ha^{-1} in 2005 and from 7 to 28 kg ha^{-1} in 2006, which corresponds to ranges of 14 to 29% and 4 to 16% of N applied with lupine seed material, respectively.

Residual SMN in the total soil profile of 120 cm depth at cabbage harvest in unfertilized control plots amounted to 40 and 26 kg N ha^{-1} in 2005 and 2006, respectively (Table 3.4). In 2006, residual SMN was significantly increased by the CM and DS-S treatment, while no effect of lupine amendments on residual SMN was observed in 2005. On average, 45 and 47% of residual SMN was recovered in the upper soil layer of 30 cm depth in 2005 and 2006, respectively.

Table 3.4 Cabbage yield parameters[z] and residual soil mineral N (SMN) in the soil layers of 0 to 30 cm and 30 to 120 cm soil depth at cabbage harvest for the unamended control (Ctrl), the coarse meal treatment (CM) and the short (DS-S) and long dense sowing (DS-L). Means of four main treatments (Table 3.1) and three replications. Different letters indicate significant differences between treatments in 2005 (lower case letters) and 2006 (capital letters) at $p \leq 0.05$ (Tukey-Kramer HSD).

	2005				2006			
	Ctrl	CM	DS-S	DS-L	Ctrl	CM	DS-S	DS-L
N_p (kg ha^{-1})	148.8c	268.3a	229.0ab	220.3b	275.1B	356.6A	364.1A	308.1B
P_{dm} (g m^{-2})	1009b	1240a	1218a	1121ab	1349B	1528A	1539A	1396B
Y_{dm} (g m^{-2})	391.4b	511.3a	513.4a	426.9b	616.8A	666.1A	674.2A	612.9A
Y_{fm} (t ha^{-1})	39.6c	56.1a	54.2ab	47.2bc	60.2B	68.4A	69.2A	61.5B
N_y (kg ha^{-1})	69.2c	120.7a	109.1ab	94.6b	149.3B	175.7A	177.3A	155.8B
SMN 0-30 cm (kg ha^{-1})	19.4a	20.6a	18.2a	16.6a	11.6B	19.0A	19.2A	15.3AB
SMN 30-120 cm (kg ha^{-1})	20.4a	19.4a	19.9a	19.6a	14.5B	19.0AB	20.5A	19.5AB

[z] N_p = total above-ground plant N; P_{dm} = total above-ground plant dry matter; Y_{dm} = cabbage head dry matter; Y_{fm} = cabbage head fresh matter; N_y = N in cabbage yield.

3.3.2 Cabbage NUE

Nitrogen use efficiency (NUE), i.e. the efficiency with which N_{av} was used to produce cabbage fresh matter yield, was generally reduced by lupine amendments (Table 3.5). Splitting NUE into individual components resulted in significant differences among treatments for all four components except for N uptake efficiency in 2006. In 2005, N uptake efficiency of fertilized cabbage was significantly higher compared to cabbage grown on unamended control plots. In both years, yield fresh matter formation was increased by lupine amendments, while N utilization efficiency for total dry matter production was reduced. No differences were observed in dry matter harvest index between lupine treatments in 2006. In contrast, in 2005 dry matter harvest index was significantly lower for the DS-L treatment compared to CM and DS-S. Generally, dry matter harvest index was higher in 2006 compared to 2005, while variation between treatments was greater in 2005. Nitrogen harvest index amounted to an average of 0.45 and 0.51 kg kg^{-1} in 2005 and 2006, respectively (Table 3.5).

Table 3.5 Nitrogen use efficiency of white cabbage, its multiplicative components[z] and N harvest index[y] for the unamended control (Ctrl), the coarse meal treatment (CM) and the short (DS-S) and long dense sowing (DS-L). Means of four main treatments (Table 3.1) and three replications. Different letters indicate significant differences between treatments in 2005 (lower case letters) and 2006 (capital letters) at p≤0.05 (Tukey-Kramer HSD).

	2005				2006			
	Ctrl	CM	DS-S	DS-L	Ctrl	CM	DS-S	DS-L
Y_{fm}/N_{av} (kg kg^{-1})	211.1a	183.0b	204.5ab	183.6b	201.6A	173.9B	172.3B	180.1B
N_p/N_{av} (kg kg^{-1})	0.788b	0.870a	0.858a	0.857a	0.912A	0.904A	0.902A	0.897A
P_{dm}/N_p (kg kg^{-1})	69.2a	46.6c	54.0b	51.9b	49.9A	43.3B	42.7B	45.9AB
Y_{dm}/P_{dm} (kg kg^{-1})	0.385bc	0.413ab	0.420a	0.379c	0.457A	0.435B	0.438AB	0.437AB
Y_{fm}/Y_{dm} (kg kg^{-1})	10.15b	10.96a	10.56a	11.02a	9.75B	10.26A	10.28A	10.07AB
N_y/N_p (kg kg^{-1})	0.461a	0.451ab	0.474a	0.427b	0.544A	0.494B	0.488B	0.505B

[z] Y_{fm}/N_{av} = N use efficiency; N_p/N_{av} = N uptake efficiency; P_{dm}/N_p = N utilization efficiency for total dry matter production; Y_{dm}/P_{dm} = dry matter harvest index; Y_{fm}/Y_{dm} = yield fresh matter formation index.
[y] N_y/N_p = N harvest index (no multiplicative component of N use efficiency).

Regression analysis of the pooled data from the two experimental years revealed significant (p≤0.05) relationships of NUE and its components with N_{av} (Fig. 3.1): Nitrogen use efficiency was found to decrease linearly with increasing N_{av} (Fig. 3.1a). Nitrogen utilization efficiency for total dry matter production was the only NUE component which was also negatively related to N_{av} following an exponential decay (Fig. 3.1c). Nitrogen uptake efficiency increased curvilinearly to an asymptotic maximum (Fig. 3.1b), while dry matter harvest index appeared to increase linearly with N_{av} (Fig. 3.1d). The latter relationship was comparatively weak. Positive linear responses of yield fresh matter formation index, i.e. the inverse of the dry matter concentration in cabbage heads, to N_{av} differed between the two years (Fig. 3.1e). The intercept of the regression line was considerably higher in 2005, while slopes were equal. Cabbage head dry matter concentration was found to be positively related to the N_t concentration in cabbage heads, in both years (Fig. 3.2). In relation to the corresponding N_t concentration, dry matter concentration was higher in 2006 than in 2005. Nitrogen use efficiency for total dry matter production was found to decrease linearly with N_{av} (Fig. 3.3).

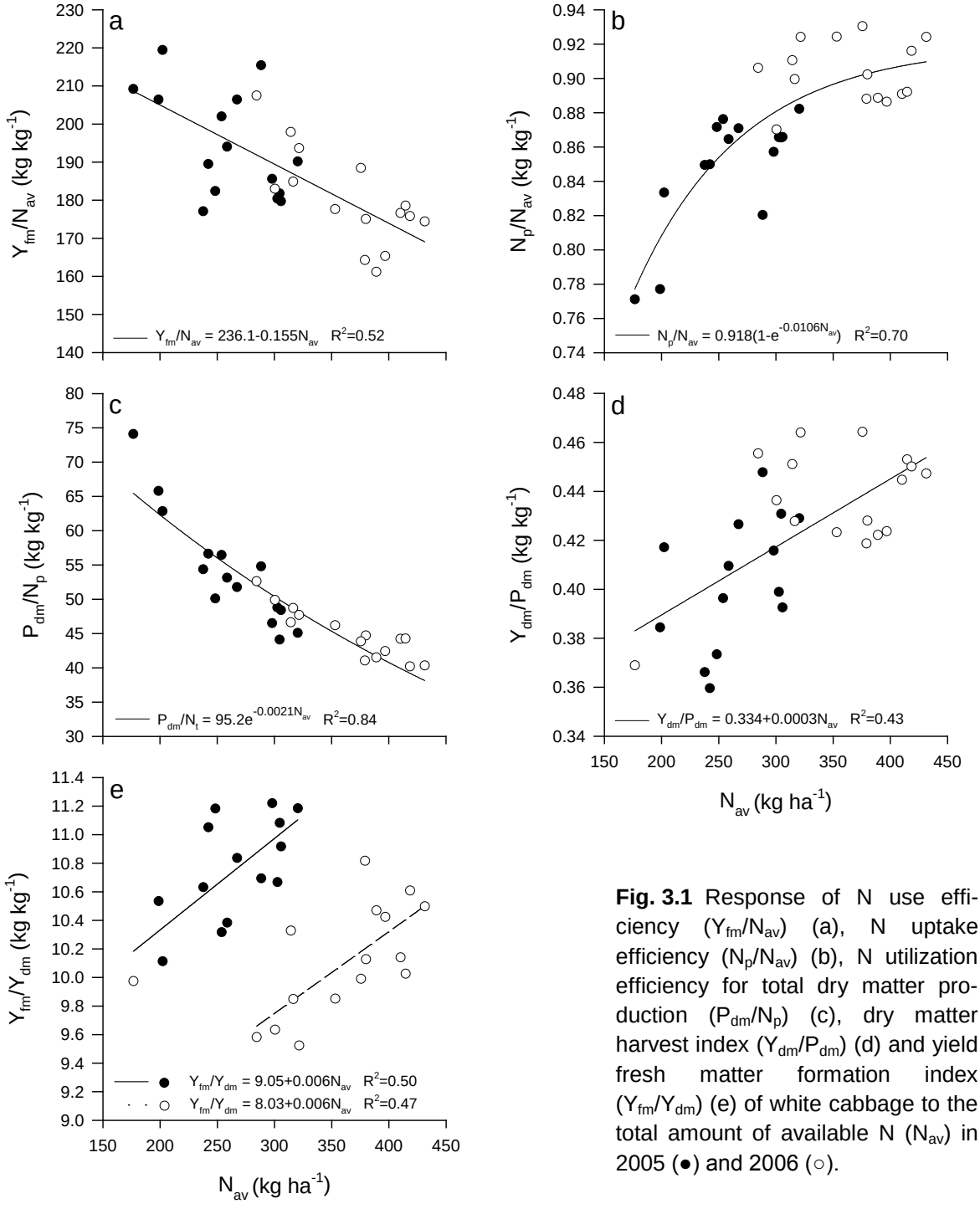

Fig. 3.1 Response of N use efficiency (Y_{fm}/N_{av}) (a), N uptake efficiency (N_p/N_{av}) (b), N utilization efficiency for total dry matter production (P_{dm}/N_p) (c), dry matter harvest index (Y_{dm}/P_{dm}) (d) and yield fresh matter formation index (Y_{fm}/Y_{dm}) (e) of white cabbage to the total amount of available N (N_{av}) in 2005 (●) and 2006 (○).

To test effects of lupine amendments on NUE and its components independently of N_{av}, an ANCOVA was carried out, whose results are reported in Table 3.6. In 2005, adjusted NUE was significantly lower for the DS-L treatment compared to DS-S. Furthermore, DS-L showed the lowest dry matter harvest index and the highest yield fresh matter formation index, both being significantly different from

DS-S. In 2006, NUE and dry matter harvest index were significantly lower for DS-L compared to the unamended control but did not differ between lupine treatments.

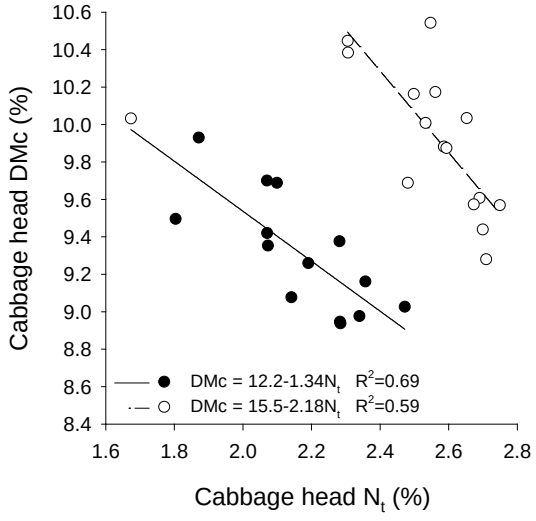

Fig. 3.2 Response of cabbage head dry matter concentration (DMc) to the total N concentration (N_t) in cabbage heads in 2005 (●) and 2006 (○).

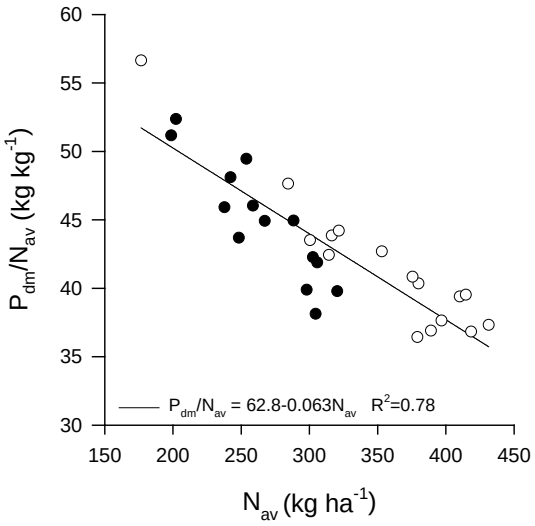

Fig. 3.3 Response of N use efficiency for total dry matter production (P_{dm}/N_{av}) to the total amount of available N (N_{av}) in 2005 (●) and 2006 (○).

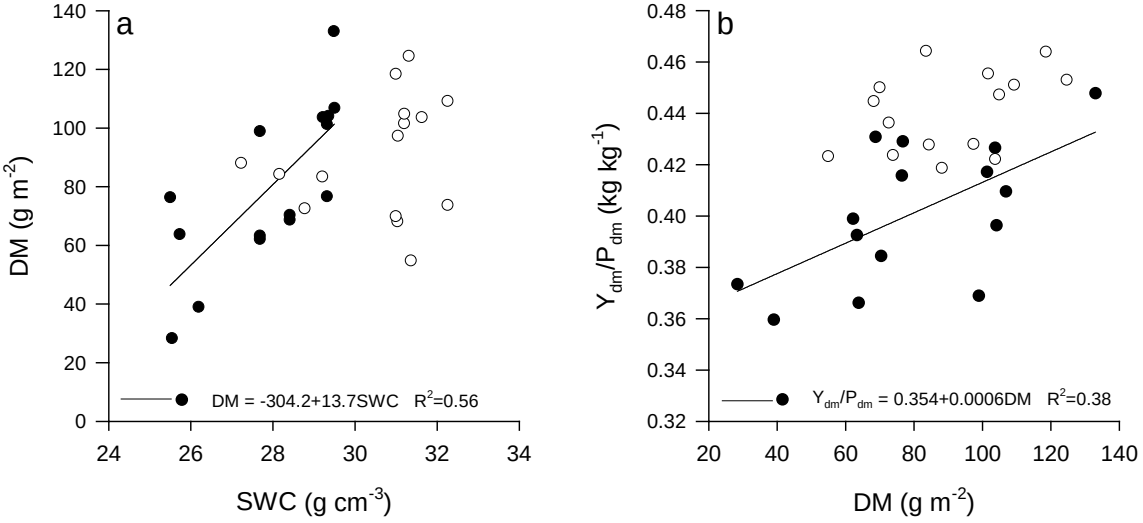

Fig. 3.4 Response of total above-ground cabbage dry matter (DM) 35 (2005) and 28 days (2006) after transplanting to the soil water content (SWC) in the soil layer of 30-60 cm depth five days before transplanting (a) and response of dry matter harvest index (Y_{dm}/P_{dm}) to respective cabbage dry matter (DM) (b) in 2005 (●) and 2006 (○).

Table 3.6 ANCOVA results for N use efficiency of white cabbage and its multiplicative components[z] for the unamended control (Ctrl), the coarse meal treatment (CM) and the short (DS-S) and long dense sowing (DS-L). Means of four main treatments (Table 3.1) and three replications adjusted for total available N (N_{av}) as covariate. Different letters indicate significant differences between adjusted means in 2005 (lower case letters) and 2006 (capital letters) at p≤0.05 (Tukey-Kramer HSD).

	2005					2006				
	Ctrl	CM	DS-S	DS-L	Slope[y]	Ctrl	CM	DS-S	DS-L	Slope[y]
Y_{fm}/N_{av} (kg kg^{-1})	197.0 ab	194.3 ab	207.5 a	183.9 b	-0.2118 *	191.5 A	179.7 AB	179.7 AB	177.1 B	-0.1703 ***
N_p/N_{av} (kg kg^{-1})	0.795 a	0.865 a	0.857 a	0.857 a	0.000102 NS	0.907 A	0.904 A	0.896 A	0.908 A	-0.00006 NS
P_{dm}/N_p (kg kg^{-1})	61.4 a	52.9 b	55.5 b	52.1 b	-0.01182 ***	45.7 A	45.8 A	44.6 A	45.6 A	-0.00720 ***
Y_{dm}/P_{dm} (kg kg^{-1})	0.401 ab	0.400 ab	0.417 a	0.378 b	0.000237 *	0.461 A	0.433 AB	0.434 AB	0.438 B	0.000078 NS
Y_{fm}/Y_{dm} (kg kg^{-1})	10.46 ab	10.71 ab	10.50 b	11.02 a	0.04691 *	10.03 A	10.10 A	10.08 A	10.16 A	0.04674 *

[z] Y_{fm}/N_{av} = N use efficiency; N_p/N_{av} = N uptake efficiency; P_{dm}/N_p = N utilization efficiency for total dry matter production; Y_{dm}/P_{dm} = dry matter harvest index; Y_{fm}/Y_{dm} = yield fresh matter formation index.
[y] ANCOVA regression slope between respective efficiency term and N_{av}; NS, *, *** = non-significant or significant at p≤0.05 or p≤0.001, respectively.

Five days before cabbage transplanting, SWC in a soil depth of 30 to 60 cm varied from 25.5 to 29.5 g cm^{-3} and from 27.2 to 32.3 g cm^{-3} in 2005 and 2006, respectively (Fig. 3.4a). In both years, SWC was lowest for the DS-L treatment (data not shown). Total dry matter of cabbage plants recorded at the first sampling about one month after transplanting was positively related to SWC in 2005 but not in 2006 (Fig. 3.4a). In turn, dry matter harvest index was found to increase significantly (p≤0.05) with early cabbage dry matter accumulation only in 2005 (Fig. 3.4b).

3.3.3 Leaching and over-winter net N mineralization

The soil water balance for the winter period 2005/2006 showed that leaching to below 120 cm did not occur, even though SWC was close to field capacity in early April 2006 (data not shown). Over-winter net N mineralization within the considered soil depth of 120 cm between cabbage harvest in 2005 and soil sampling in spring 2006 increased linearly with the amount of N in cabbage harvest residues by 0.63 kg kg^{-1} (Fig. 3.5).

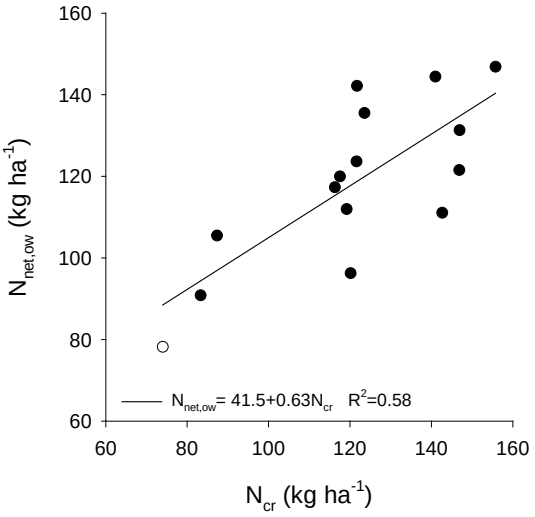

Fig. 3.5 Relationship between the residual amount of N in cabbage harvest residues (N_{cr}) in 2005 and over-winter net N mineralization ($N_{net,ow}$).

3.3.4 Beetroot N supply and yield

Since no additional N fertilizer was applied, N_{av} for beetroot was simply composed of SMN already present in spring 2006 and N mineralized from SOM between spring soil sampling and beetroot harvest. The latter was not found to be enhanced by any of the lupine amendments of the previous year (Table 3.7). Considering the upper soil layer of 60 cm depth, spring SMN of the CM treatment was significantly higher than the unamended control. Spring SMN in the lower soil layer of 60 to 120 cm depth was increased by the CM and DS-S treatment. Total N_{av} for beetroot of all three lupine amendments was significantly higher compared

Table 3.7 Amount of total available N (N_{av}) for beetroot as composed of spring soil mineral N (SMN) in the soil layers of 0 to 60 cm and 60 to 120 cm depth and net N mineralization from soil organic matter (SOM) during beetroot growth period and beetroot fresh matter yield (Y_{fm}) for the unamended control (Ctrl), the coarse meal treatment (CM) and the short (DS-S) and long dense sowing (DS-L). Means of four main treatments (Table 3.1) and three replications. Different letters indicate significant differences between treatments at $p \leq 0.05$ (Tukey-Kramer HSD).

	Ctrl	CM	DS-S	DS-L
N_{av} (kg ha^{-1})	202.3 b	240.5 a	232.9 a	228.8 a
Spring SMN 0-60 cm (kg ha^{-1})	101.8 b	134.4 a	122.9 ab	124.2 ab
Spring SMN 60-120 cm (kg ha^{-1})	26.1 c	41.6 a	35.0 ab	31.7 bc
SOM net N mineralization (kg ha^{-1})	74.4 a	64.5 a	75.0 a	72.9 a
Y_{fm} (t ha^{-1})	36.5 a	39.5 a	38.2 a	37.5 a

to the control treatment. However, no significant differences in N_{av} between lupine treatments were observed. The increase in N_{av} due to lupine amendments averaged 32 kg ha^{-1}, which corresponds to 18% of lupine seed N applied in the previous year (data not shown). The same ranking of treatments as observed for N_{av} was found for beetroot fresh matter yield (Table 3.7). However, yield differences were not statistically significant.

3.4 Discussion

3.4.1 Cabbage NUE

According to our expectations, NUE of white cabbage was found to decrease with increasing N_{av}. Similar observations were reported also for other vegetable crops including bell pepper (Olsen et al. 1993; Van Eerd 2007), bunching onion (Inal and Tarakcioglu 2001) and rocket salad (Santamaria et al. 2002). The analyses of individual components of NUE revealed that, in the present case, the negative NUE response to N_{av} was solely due to a decrease in N utilization efficiency for total dry matter production. All other NUE components were found to be positively related to N_{av}. Since N utilization efficiency for total dry matter production represents the inverse of the N_t concentration in above-ground cabbage dry matter, the decrease in NUE was based on an increasing N_t concentration, indicating that cabbage N uptake increased stronger with N_{av} than dry matter production. A higher N_t concentration in *Brassica* vegetables was frequently reported to be accompanied by lower dry matter concentration (Booij 2000; Everaarts and Booij 2000; Everaarts and De Moel 1998; Freyman et al. 1991; Peck 1981; Schulte auf'm Erley et al. 2010). This observation was confirmed in the present study and was the cause for the positive response of yield fresh matter formation index to N_{av}. Thus, the negative effect of an increasing N_t concentration on NUE is lowered by an accompanied increase in the water content of cabbage heads. Higher fresh matter yield due to higher N supply is therefore not only based on higher dry matter production but also on a higher proportion of water in the produce.

Initially surprising, N uptake efficiency was lowest at low N_{av} and increased to an asymptotic maximum. However, this observation is explained by the fact that at the generally low N supply in 2005 residual SMN was unaffected by N_{av}, amounting to an average of 38 kg ha^{-1} in the total soil profile of 120 cm depth. Due to its character as a ratio, N uptake efficiency will be low, if N_{av} is low and as consequence a comparatively large proportion of N_{av} remains in the soil as residual SMN. Roots of white cabbage were reported to be in contrast to sweet corn and carrot evenly horizontally distributed (Kristensen and Thorup-Kristensen 2004). However, the inevitable minimum amount of residual SMN can, at least partly, be explained by an inhomogeneous distribution of both SMN and roots, which does not allow for an entire depletion of SMN even at suboptimal N supply (Schenk 1998). Furthermore, root length density of vegetable crops in general in the upper layer of 30 cm soil depth typically ranges between 2 and 4 cm cm^{-3}, which is low in comparison to cereals (Schenk 1998). In the present study, residual SMN after cabbage harvest was to a large proportion concentrated in this soil layer. Everaarts and Booij (2000) as well as Thorup-Kristensen and Sørensen (1999) made similar observations.

Nitrogen *use* efficiency for total dry matter production, i.e. the efficiency with which N_{av} was used to produce total cabbage dry matter, represents the product of N uptake efficiency and N *utilization* efficiency for total dry matter production. Despite the curvilinear response of its two components to N_{av}, a strong negative linear relationship (R^2=0.78) between N use efficiency for total dry matter production and N_{av} was found (Fig. 3.3). In contrast, the dependency of NUE on N_{av} was of only moderate strength (R^2=0.52). This can be attributed to the high variation in dry matter harvest index, which was only weakly related to variation in N_{av} (Fig. 3.1d).

The harvest index of white cabbage typically increases with growing time (De Moel and Everaarts 1990). Using a base temperature of 10°C, Isenberg et al. (1975) quantified the thermal time required by late-maturing white cabbage to reach harvest maturity to be approximately 1000°Cd. The generally lower harvest index in 2005 is therefore rather due to the shorter thermal growing period of 782°Cd compared to 1027°Cd (10°C base) in 2006 than to lower N_{av}. Cabbage

maturation in the field is additionally characterized by an accumulation of total solids and consequently by an increase in dry matter concentration (Suojala 2003). Wennberg et al. (2002) quantified dry matter concentration of cabbage heads to increase from 8.6 to 9.4% during the last three weeks before harvest. However, this effect was not consistent across various varieties. Differences in maturity between the two experimental years are therefore strengthened by the comparatively higher dry matter concentration of cabbage heads harvested in 2006.

Effects of temperature sum do not explain variation in harvest index within each year. Variation was more pronounced in 2005, with the DS-L treatment showing the lowest harvest index. By adjusting NUE for the influence of N_{av} (ANCOVA), the low harvest index of the DS-L treatment is also reflected in a strikingly low NUE. Low harvest indices were found to be associated with low water availability during early cabbage growth, which was probably caused by transpiration losses due to developing lupine seedlings. This is in general accordance with observations of Imtiyaz et al. (1999), who reported that harvest index of white cabbage, grown in a semi-arid climate, declined gradually with decreasing amount of irrigation water. Irrigation treatments started two weeks after transplanting and lasted until harvest. This is in sharp contrast to the present study, in which effects of lupine seedlings on cabbage water supply were strongest at transplanting and leveled off during the early phase of cabbage growth. Similar to our finding of a positive harvest index response to early crop growth, Schulte auf'm Erley et al. (2010) reported that a low harvest index was related to a slow leaf emergence at early growing stages caused by low temperatures. Thus, as discussed above for the general difference in harvest index between 2004 and 2005, a low harvest index is associated with an early maturity stage which, in turn, can result from a retarded crop development. This is once more supported by the fact that the low harvest index of DS-L in 2005 was accompanied by a high ANCOVA-adjusted yield fresh matter formation index (Table 3.6), which represents a comparatively low cabbage head dry matter concentration. Variation in early growth can therefore explain why attempts to predict maturity date by accumulated heat units have been of only limited success (Radovich et al. 2004; Strandberg and White

1979). The number of days from transplanting to cabbage harvest was also shown to decrease with increasing plant density (Stoffella and Fleming 1990). This was interpreted by Wien and Wurr (1997) to indicate that maturation is influenced by the level of resource availability, which becomes increasingly limited during crop growth. Our results suggest that the date of cabbage maturity is affected in much earlier growth stages than presently assumed. While in the present study early growth was hampered by low water availability, early cabbage growth may in general be impacted by a number of factors including the quality of transplants, transplanting technique, nutrient availability as well as disease and pest infestation.

3.4.2 Residual effects

After harvest of white cabbage, large amounts of N remain in the field in residues. The observed N harvest indices were within the range of 40 to 60% reported in previous studies (Everaarts and Booij 2000; Haas et al. 2007; Peck 1981). In total, only 4 to 29% of applied lupine seed N was removed with cabbage yield, while the larger fraction was either not mineralized during cabbage growth or remained in crop residues. Remaining fertilizer N led to a significant increase in the N supply for the subsequent beetroot crop by on average 18% of lupine seed N applied in the previous year. Similarly, Riley et al. (2003) investigated the residual effect of chopped grass and clover material, which was surface applied to white cabbage and beetroot, and recovered up to 6% of applied mulch N in above-ground biomass of a subsequent barley crop. However, it remained unclear whether this N increment derived from residual SMN in previous autumn, from vegetable residues or from further mineralization of mulch material. In the present study, net N mineralization from SOM during beetroot growth period was increased by none of the previous lupine treatments. Thus, the observed differences in beetroot N supply were due to differences in SMN already present in spring. The observed slope of the linear regression between cabbage residue N and over-winter net N mineralization represents an average net N mineralization from cabbage residues of 63%. This is in close agreement with results obtained by aerobic incubation of various *Brassica* vegetable residues (De Neve and Hofman 1996), showing that

maximum net N mineralization calculated by a first order kinetic model averaged 67% (range 60-78%) and 44% (range 35-55%) for leaves and stems, respectively. Thus, even a marginal N release from lupine seeds during the winter period cannot be ruled out entirely, present results indicate that the by far larger residual effect potentially derives from the incremental fertilizer N in *Brassica* residues. This observation is based on a single application of a comparatively easily decomposable organic fertilizer. It is therefore not in contrast with other reports suggesting that a regularly repeated application of particularly more slowly decomposing organic amendments can considerably increase N mineralization from SOM (Gutser et al. 2005; Indraratne et al. 2009; Peu et al. 2007).

3.5 Conclusions

Besides its negative response to available N, NUE of cabbage can be considerably affected by agronomic practices others than those influencing N availability. Suboptimal crop establishment significantly delays cabbage maturation, which could prevent particularly late-maturing cultivars from reaching maturity within the given growth period. As premature cabbage harvest is associated with a low harvest index, optimal early growth can be crucial for realizing the full yield potential and consequently for ensuring high NUE. Under drought stress conditions or in case of short thermal growth periods, the use of earlier-maturing cabbage cultivars is recommendable to obtain high NUE by timely maturity.

The efficiency with which N applied with easily mineralizable organic fertilizers is used within *Brassica* rotations depends largely on the net N mineralization of the fertilizer within the year of application as well as on the re-utilization of incremental fertilizer N in *Brassica* residues by the succeeding crop. Both should be considered in the economic evaluation of organic fertilizer applications. The risk of N leaching losses after organically fertilized cabbage derives in the first instance from harvest residues and secondly from residual SMN at cabbage harvest, while potential leaching losses due to late mineralizing organic fertilizer N are expected to be marginal.

4 Narrow-leaved lupine as an N source alternative to grass-clover swards in organic vegetable rotations

Abstract

Legumes represent an important N source in organic vegetable rotations. Since neither the amount of N_2 fixed nor N mineralization from legume biomass can be influenced satisfactorily and since both is highly variable, availability of N for following crops does often not match their requirements. The production of grain legume seeds followed by their temporary storage and reallocation as N fertilizer may represent a flexible and well controllable alternative to traditional legume green manures.

In two field experiments on a Typic Hapludalf soil, the use of narrow-leaved lupine (Lupinus angustifolius L.) as an N source in organic field vegetable production was investigated and compared to that of two grass-clover treatments, in which cut herbage was either removed from the sward or remained as mulch. Legume precrops were followed by white cabbage and beetroot in the second and third crop rotation year, respectively.

Symbiotic N_2 fixation of legume crops was highest for cut grass-clover swards, averaging about 350 kg ha^{-1}. Mulching reduced N_2 fixation considerably, on average by 57%. Except for lupine crops affected by pigeon feeding, lupine N_2 fixation was largely comparable to that of mulched grass-clover swards. Net N mineralization from grass-clover residues within the year of their incorporation was positively related to the percentage of clover in the mixture (R^2=0.93), varying with sward management and experimental year. Application of coarsely milled lupine seeds on plots previously cropped with lupines resulted in an N supply for white cabbage either not differing from cabbage N supply after mulched grass-clover or being higher. Due to the export of N with removed grass-clover herbage, the N benefit of cut swards was insufficient for white cabbage in both experiments. Residual effects on N supply for beetroot averaged -2 and 28 kg N ha^{-1} for lupine and grass-clover, respectively, indicating that the N benefit from lupine crop residues and coarsely milled seeds is rather short-lived, while ploughing of grass-clover swards has a positive longer-term effect on net N mineralization from soil organic matter. From potential N leaching losses after legume precrops it is concluded that lupines provide a viable N source alternative to mulched grass-clover swards on sites with either low N leaching risk or low to moderate mineralization potential of indigenous soil organic N.

4.1 Introduction

Because of their ability to fix dinitrogen (N_2) from the atmosphere, legumes represent an important nitrogen (N) source in organic agro-ecosystems. A traditional way of making use of this N source is the integration of frequently cut grass-clover swards into the crop rotation. Ploughing of swards results in the net mineralization of a part of the N accumulated in above- and below-ground sward biomass and thereby increases the N availability for succeeding crops (Davies et al. 2001; Gunnarsson et al. 2008; Hauggaard-Nielsen et al. 1998; Høgh-Jensen and Schjoerring 1997). Variation in net N mineralization from green manure and crop residues can largely be explained by their C:N ratio or total N (N_t) concentration (Chaves et al. 2004; Seneviratne 2000; Trinsoutrot et al. 2000).

A main disadvantage of legume green manuring in general is, however, that the amount of N_2 fixed cannot be influenced satisfactorily. Moreover, the average N_t concentration of grass-clover swards depends largely on the percentage of clover in the mixture, as the N_t concentration of clover biomass is generally higher than of the grass component (Huss-Danell et al. 2007). Clover percentage is reported to vary with inorganic soil N (Templeton and Taylor 1966), time of the year (Elgersma and Hassink 1997), sward management (Hatch et al. 2007) and cutting frequency (Høgh-Jensen and Kristensen 1995). As a consequence, the amount of available N provided by traditional legume green manures is highly variable and does often not match the N demand of the following crop (Båth 2000; Crews and Peoples 2005).

Coarsely milled lupine seeds have been previously evaluated as valuable N fertilizer for organic horticulture (Chapter 2; Müller and von Fragstein und Niemsdorff 2006a; 2006b; Stadler et al. 2006). They can be produced by farmers themselves and reallocated after milling in well-controlled amounts flexibly in time and space. Studies on the biochemical composition of lupine seeds revealed that their crude protein content ($N_t \times 6.25$) is largely controlled by lupine genotype and is usually stable over a wide range of environmental conditions (Bhardwaj et al. 1998; Bhardwaj et al. 2004; Carvalho et al. 2005). Stability in seed chemical

characteristics, which determine their potential net N mineralization, assures a reliable N fertilizer effect of locally produced lupine seeds.

At crop maturity, a large proportion of the amount of N_2 fixed by lupines is located in the seeds. Jost (2003) estimated field N balances of two varieties of white lupine (*Lupinus albus* L.), i.e. fixed N_2 minus N in harvested seeds, grown in three seasons and at four locations in Northern Germany. The average N balance amounted to +6 kg ha^{-1} indicating that, on average, N_2 fixation only slightly exceeded the amount of N in seed yield. If rhizodeposition of N during lupine crop growth was additionally taken into account when calculating N_2 fixation, the average N balance increased to +30 kg ha^{-1}.

Thus, the production of lupine seeds followed by their temporary storage and reallocation as coarse meal would combine the possibility of utilizing legume N_2 fixation as local N source with the high flexibility and controllability of commercial organic fertilizers. This will, as a consequence, allow for matching the availability of N closely with crop requirements.

In the present study, the use of narrow-leaved lupine (*Lupinus angustifolius* L.) as N source in organic field vegetable production was investigated and compared to that of grass-clover swards. Criteria for evaluation and comparison of legume systems included the N input by symbiotic N_2 fixation and their ability to provide N to succeeding crops. As these factors were expected to be influenced by management practices and genotype of grass-clover and lupine, respectively, precrops included two grass-clover treatments, in which cut herbage was either removed from the sward or remained as mulch, and two lupine varieties differing in growth habit and alkaloid content.

4.2 Materials and methods

4.2.1 Study site, experimental setup and agronomic management

Two crop rotation experiments with identical design and treatments were initiated in 2004 (Exp. I) and 2005 (Exp. II) on organically managed fields at the university's experimental station in Ruthe, situated 15 km south of Hannover (52°14' N, 9°48' E). The soil was a Typic Hapludalf (FAO classification: Haplic Luvisol) developed from Weichselian loess deposits (>1.2 m) over Quaternary sand and gravel with approximately 10% sand, 80% silt and 10% clay. Soil organic matter content in the plough layer of 30 cm depth amounted to 1.4%.

In general, each experiment comprised an initial season with different precrop treatments followed by one (Exp. I) and two (Exp. II) succeeding vegetable crops. Experiments were arranged in a randomized complete block design with three replicates. Precrops included two narrow-leaved lupine genotypes (*Lupinus angustifolius* L. cv. 'Boruta', cv. 'Azuro'), two differently managed grass-clover mixtures and spring wheat (*Triticum aestivum* L. cv. 'Thasos'). Lupine varieties differed in growth habit and alkaloid content, with 'Boruta' being a low alkaloid and restricted branching phenotype and 'Azuro' being a high alkaloid type with indeterminate growth pattern. The realized plant density averaged 61 and 86 plants m^{-2} for 'Boruta' and 'Azuro', respectively. Grass-clover was composed of white clover (*Trifolium repens* L. cv. 'Rivendel'), red clover (*Trifolium pratense* L. cv. 'Titus') and perennial ryegrass (*Lolium perenne* L. cv. 'Lipondo') with approximate proportions of 10, 30 and 60% (w/w) of the seed mixture, respectively. Grass-clover was mown three times during the first year and once again the following spring. Cut herbage was either removed (CT) or mulched (MU). At the first mowing, herbage was removed from plots of both treatments to assure a proper initial development of the clover fraction also in the MU treatment. Thus, grass-clover treatments differentiated from the second mowing event on. Additionally, pure swards of perennial ryegrass (*L. perenne* L. cv. 'Edda') were established, which were cultivated as the grass-clover CT treatment. Prior to sowing, lupine seeds were inoculated with lupine-nodulating *Bradyrhizobium* bacteria (Radicin No. 6, Jost GmbH, Iserlohn, Germany). The grass-clover seed

mixture was sown without prior inoculation as the experimental fields were previously cropped with ryegrass, which contained a small but evenly distributed amount of clover when moldboard ploughed in 2003. Lupines and wheat were harvested using a Hege 140 (Wintersteiger AG, Ried im Innkreis, Austria) plot combine. Residues were incorporated shortly after crop harvest by chisel (Exp. I) and moldboard ploughing (Exp. II). Grass-clover swards were moldboard ploughed the following spring.

Precrops were followed by white cabbage (*Brassica oleracea* L. convar. *capitata* var. *capitata* f. *alba* cv. 'Impuls'), which was planted at a density of 4.3 plants m^{-2}. Five days prior to cabbage planting, lupine plots were divided into subplots, which were either amended with coarsely milled lupine seeds or kept unamended. Lupine seeds used for fertilization were obtained from precrops in 2004. Lupine coarse meal was applied in amounts of 3.8 t ha^{-1} corresponding to 180 kg N ha^{-1} and was incorporated with a rotary tiller. In Exp. I, considerable amounts of weed biomass were removed from cabbage plots by hand weeding at the end of July. Weed samples were taken shortly prior to weeding to record the amount of N removed with weeds. Following hand harvest of cabbage heads, residues were

Table 4.1 Selected dates of agronomic management events and samplings

	Exp. I	Exp. II
Initial soil sampling	Apr 1, 2004	Apr 19, 2005
Sowing of precrops and ryegrass	Apr 14, 2004	Apr 21, 2005
Lupine interim sampling	July 7, 2004	July 18, 2005
Harvest of spring wheat	Aug 23, 2004	Aug 16, 2005
Harvest of lupines	Sep 6/7, 2004	Aug 29-31, 2005
Last (4^{th}) mulching/cutting of grass-clover	May 2, 2005	May 9, 2006
Spring soil sampling before cabbage	June 3, 2005	May 31, 2006
Application of lupine coarse meal	June 3, 2005	June 1, 2006
Incorporation of grass-clover swards	June 6, 2005	June 1, 2006
Planting of cabbage	June 8, 2005	June 6, 2006
Harvest of cabbage	Oct 17, 2005	Oct 10, 2006
Incorporation of cabbage harvest residues	Nov 8, 2005	
Sowing of beetroot	June 27, 2006	
Harvest of beetroot	Sept 20, 2006	

incorporated by moldboard ploughing. A beetroot (*Beta vulgaris* L. var. *conditiva* cv. 'Detroit 2/Bolivar') crop with an average plant density of 35 plants m^{-2} was established in 2006 following cabbage grown in 2005 (Exp. I). Selected dates of agronomic management events are indicated in Table 4.1.

4.2.2 Sampling and chemical analysis

Soil samples were collected to a depth of 120 cm in four layers of 30 cm each. Soil mineral N (SMN) was extracted by addition of 0.1 N KCl solution. The extract was analyzed for NO_3^- and NH_4^+ using an UV spectrometer (Lambda 2S, PerkinElmer Inc., Waltham, MA, USA).

Above-ground plant samples of grain crops, grass(-clover) swards and vegetable crops were taken from sampling areas of on average 1.2, 0.6 and 1.8 m² per plot, respectively. Sampling dates are given in Table 4.1. Samples taken at harvest of grain and vegetable crops were separated into yield and non-yield biomass. Grain yield was additionally determined by combine harvest. The difference between seed yield determined by plant sampling and combine harvest was attributed to seed losses during combine harvest. Lupine samples were additionally separated into a leaf fraction. Mulch material in previously mulched grass-clover swards was sampled separately by manual collection. Plant samples were dried at 70°C until weight constancy and were analyzed for N_t by the micro-Kjeldahl procedure including nitrate reduction to ammonia or alternatively by the Dumas method using an elemental analyzer (Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany).

4.2.3 Data analysis

4.2.3.1 Estimation of below-ground N

Below-ground N of precrops was not measured directly by root sampling but was estimated using literature reported root/shoot ratios. For clover (*T. pratense* L. and *T. repens* L.) in pure stand and in mixture with ryegrass an average ratio of root N to accumulated above-ground N of 29% (published range: 11-42%) was derived from literature data (Badaruddin and Meyer 1989; 1990; Bruulsema and Christie

1987; Groya and Sheaffer 1985; Huss-Danell et al. 2007; Kirchmann 1988; Kumar and Goh 2000; 2002; Loges 1998; Rasmussen et al. 2007) and used to estimate below-ground N of grass-clover swards at incorporation date. The corresponding value for pure ryegrass was 55% (range: 48-63%, Kumar and Goh 2002; Loges 1998). The root N to shoot N ratio of agronomically relevant lupine species at maturity including *L. albus*, *L. angustifolius* and *L. luteus* was reported to range between 4 and 8% (Jost 2003; Unkovich et al. 1994), averaging 6%. In all studies cited above, quantification of root N was determined by conventional root excavation followed by separation-washing. Studies in which below-ground N was measured using ^{15}N-labeling techniques were not considered here. Respective data is comparatively scarce in literature and mixing up of principally different methods was intended to be avoided, since ^{15}N-labeling techniques generally result in considerably higher below-ground N estimates (Khan et al. 2002; McNeill and Fillery 2008; Russell and Fillery 1996).

4.2.3.2 Symbiotic N_2 fixation

Symbiotic N_2 fixation of lupine and grass-clover precrops at harvest and at incorporation date, respectively, was estimated according to an extended N difference method (Hauser 1992). The difference between the total amounts of N (kg ha^{-1}) recovered at precrop end in the legume ($N_{LEG\ tot}$) and in a non-leguminous reference crop ($N_{REF\ tot}$) was attributed to N_2 fixation (N_{fix}):

$$N_{fix} = N_{LEG\ tot} - N_{REF\ tot} \qquad [4.1]$$

Perennial ryegrass was used as reference for both lupine and grass-clover. The total N amount of ryegrass was calculated as

$$N_{REF\ tot} = N_{REF\ shoot} + N_{REF\ root} + SMN_{REF} + N_{REF\ cut} - N_{REF\ seed}\ , \qquad [4.2]$$

where $N_{REF\ shoot}$ and $N_{REF\ root}$ represent above- and below-ground ryegrass N, respectively, SMN_{REF} is the amount of soil inorganic N to a depth of 120 cm, $N_{REF\ cut}$ represents previously cut and removed above-ground N and $N_{LEG\ seed}$ is the N amount applied with seeds. Similar to $N_{REF\ tot}$, $N_{LEG\ tot}$ of cut and mulched grass-clover swards was computed as

$$N_{LEG\ tot} = N_{LEG\ shoot} + N_{LEG\ root} + SMN_{LEG} + N_{LEG\ cut} - N_{LEG\ seed}\ , \qquad [4.3]$$

where, in case of mulched grass-clover, $N_{LEG\ cut}$ represents the amount of N recovered in mulch material at sward incorporation date. Lupine $N_{LEG\ tot}$ was quantified as

$$N_{LEG\ tot} = N_{LEG\ shoot} + N_{LEG\ root} + SMN_{LEG} + N_{LEG\ leaf\ lit.} - N_{LEG\ seed}\ , \qquad [4.4]$$

where $N_{LEG\ leaf\ lit.}$ represents the amount of N in leaf litter, i.e. in lupine leaves fallen before crop harvest. Leaf litter N was calculated by multiplying leaf litter dry matter by an exemplarily determined N_t concentration of 2.1%. Leaf litter dry matter was obtained as difference between remaining leaf dry matter at crop harvest and the maximum amount of leaf dry matter determined in interim samplings conducted shortly prior to the onset of intense leaf fall.

4.2.3.3 Leaching

To account for potential N losses during the two winter leaching periods, soil water balances were computed using weather data recorded at the experimental station. The reference evapotranspiration ET_0 was obtained by the FAO Penman-Monteith equation (Allen et al. 1998) on a daily basis using maximum and minimum values of temperature and relative humidity, daily average wind speed and solar radiation as input parameters. The actual evapotranspiration ET_c was obtained by multiplying ET_0 by the crop specific coefficient K_c. For grass-clover swards a K_c coefficient of 0.95 was used and derived by averaging K_c coefficients for cut berseem clover (*Trifolium alexandrinum* L.) and ryegrass presented by Allen et al. (1998). To calculate the actual evaporation from bare soil after harvest of lupine, wheat and cabbage the dual crop coefficient approach was applied, in which K_c is split into the basal crop coefficient K_{cb} and the evaporation coefficient K_e (Allen et al. 1998). Since K_{cb} primarily represents the transpiration component, K_{cb} was set to zero by reason of the lack of plants. The evaporation coefficient K_e was computed on a daily basis depending on the total amount of water being able to evaporate from the soil surface layer of 10 cm (Allen et al. 1998). The amount of leachate draining to below 120 cm soil depth was derived by consideration of

the initial amount of soil water present in autumn, the soil specific field capacity with respect to a soil depth of 120 cm and the computed soil water balance.

4.2.3.4 Available N for vegetable crops

The total amount of available N (N_{av}) for white cabbage and beetroot during the growing season was quantified by summing above-ground total plant N, residual SMN at harvest and N removed with weed biomass during crop growth. Net N mineralization of lupine coarse meal was calculated as difference in N_{av} between amended soils and unamended control soils. Net mineralization of N from soil organic matter (SOM) during cabbage growth period was derived from unamended soils and computed as difference between SMN at cabbage planting and N_{av} at cabbage harvest. Net N mineralization from SOM of wheat plots was used as a reference to quantify net N mineralization of grass-clover residues.

The precrop N effect of lupines (PE_{lup}) on white cabbage was calculated as difference in cabbage N_{av} between lupine plots not amended with coarse meal ($N_{av,lup}$) and plots previously cropped with spring wheat ($N_{av,sw}$):

$$PE_{lup} = N_{av,lup} - N_{av,sw} \qquad [4.5]$$

The residual N effect of legume precrops (RE_{leg}) was defined as increment in SOM net N mineralization after cabbage harvest in reference to spring wheat and was computed as

$$RE_{leg} = SOMN_{net,leg} - SOMN_{net,sw}\ , \qquad [4.6]$$

where $SOMN_{net,leg}$ and $SOMN_{net,sw}$ represent the net N mineralization from SOM in the period between cabbage and beetroot harvest for legume and spring wheat precrops, respectively. To allow for differentiation between N mineralized from SOM and from cabbage harvest residues, net N mineralization of residues was estimated to be 63% as determined in simultaneous experiments carried out at the same location (see Chapter 3.3.3).

4.2.3.5 N budgets and comparison of legume systems

The precrop N balance was derived by subtracting the N amount removed with harvest material from N_{fix}. For each entire crop rotation, a simple N balance was calculated in consideration of precrop N balance, N input by application of lupine coarse meal and N output due to N removed with cabbage and beetroot yield biomass.

Lupine coarse meal (CM) was applied in amounts of 180 kg N ha^{-1} irrespective of the amount of lupine N yield (LNY) in the previous year. In order to establish comparability in cabbage N_{av} between lupine and grass-clover precrops, the net N amount mineralized from lupine coarse meal ($N_{net,CM}$) during cabbage growth period was corrected for LNY (kg ha^{-1}). Assuming that the percentage of net N mineralized from CM ($r_{N,CM}$) is not affected by the amount of CM applied, $N_{net,CM}$ (kg ha^{-1}) was calculated as follows:

$$N_{net,CM} = LNY \times r_{N,CM} \times 0.01 \quad [2.7]$$

Corrected cabbage N_{av} of lupine treatments was computed as sum of spring SMN, net N mineralization from SOM during cabbage growth period and $N_{net,CM}$ and represents the potential N_{av} being achieved if the amount of N applied with coarse meal equals LNY.

4.2.3.6 Statistics

Data from Exp. I and Exp. II were analyzed conjointly by applying multifactorial analysis of variance (ANOVA) using SAS's PROC GLM procedure (SAS 9.1, SAS Institute Inc., Cary, NC, USA) with *precrop*, *experiment* and *block* (nested within *experiment*) as factors. Only Exp. I comprised a third crop rotation year. Consequently, the statistical model was reduced by the factor *experiment* when analyzing data sets solely available for Exp. I. Following a significant ANOVA result for the factor *precrop* ($p \leq 0.05$), separation of precrop means was performed by Fisher's LSD test at a significance level of $p \leq 0.05$. Percentage data were arcsin-transformed prior to analysis as far as data sets did not include negative values or values greater than 100% making transformation impossible.

4.3 Results

4.3.1 Initial soil mineral N status

Indigenous soil N supply for precrops differed considerably between the two experiments, which is reflected in the total N uptake of spring wheat amounting to 72 kg ha^{-1} in Exp. I compared to 127 kg ha^{-1} in Exp. II (Table 4.2). A difference of similar magnitude was already found in initial SMN before sowing of precrops, amounting to 30 and 109 kg ha^{-1} in 0-60 cm soil depth in Exp. I and II, respectively (data not shown).

Table 4.2 Total N uptake, N remaining in residues, N removed with yield biomass and symbiotic N_2 fixation of lupines, mulched (MU) and cut (CT) grass-clover and spring wheat

	Precrop	N uptake	Residue N	N yield	N_2 fixation
		kg ha^{-1}			
Exp. I	Lupine 'Boruta'	167.6[z]	66.9[x]	100.8	88.1
	Lupine 'Azuro'	297.1[z]	140.3[x]	156.9	211.9
	Grass-clover MU	608.1[y]	323.6[w]	28.5	253.3
	Grass-clover CT	613.3[z]	225.8[v]	387.5	469.5
	Spring wheat	71.9[z]	19.2[u]	52.7	-
Exp. II	Lupine 'Boruta'	185.9[z]	72.8[x]	113.0	91.2
	Lupine 'Azuro'	185.0[z]	86.7[x]	98.2	87.3
	Grass-clover MU	409.3[y]	228.8[w]	72.3	73.8
	Grass-clover CT	448.4[z]	130.4[v]	318.0	228.5
	Spring wheat	127.4[z]	33.5[u]	93.9	-
ANOVA	Precrop	***	***	***	***
	Exp.	***	***	NS	***
	Precrop x Exp.	***	**	***	**
Fisher's LSD ($p \leq 0.05$)	Precrop, Exp. I	65.7	29.9	39.1	123.8
	Precrop, Exp. II	91.5	69.3	31.2	79.4

NS, **, *** = non-significant or significant at $p \leq 0.01$ or $p \leq 0.001$, respectively; n=3.

[z] = Residue N + N yield

[y] = Residue N + N yield + N in mulched herbage not recovered at incorporation

[x] N in vegetative above- and below-ground lupine biomass, pod walls and leaf litter at harvest and in seeds lost during combine harvest.

[w] N in living above- and below-ground grass-clover biomass and in recovered mulch material at incorporation.

[v] N in living above- and below-ground grass-clover biomass.

[u] N in vegetative above- and below-ground wheat biomass at harvest and in seeds lost during combine harvest.

4.3.2 N leaching losses

Soil water balances for winter periods 2004/2005 and 2005/2006 showed that leaching to below 120 cm did not occur, even though soil water content was temporarily close to field capacity in spring 2005 and 2006 (data not shown). Therefore, N losses due to leaching had not to be considered in the following.

4.3.3 Legume growth performance and N_2 fixation

Grass-clover

At sward incorporation, total N concentration of above-ground biomass varied between 2.9 and 3.9% and was closely related the content of clover in the mixture (Fig. 4.1a). Clover content was generally higher in Exp. I than in Exp. II and was considerably reduced by mulching in both experiments. Total N uptake of swards was higher in Exp. I but was not found to be significantly affected by sward management practices (Table 4.2). Nitrogen yield of cut swards amounted to an average of 353 kg ha^{-1}. Nitrogen yield of mulched swards, i.e. N removed at the first mowing event, averaged 50 kg ha^{-1} and was more than twice as high in Exp II than in Exp. I. Mulching increased the amount of N in residues, i.e. N incorporated with sward biomass, by 98 kg ha^{-1} in both experiments. Symbiotic N_2 fixation was highest for cut swards, averaging 349 kg ha^{-1}. Mulching reduced N_2 fixation considerably by on average 57%.

Lupine

Dinitrogen fixation of lupines averaged 120 kg ha^{-1} and was either comparable to or lower than N_2 fixation of mulched grass-clover swards (Table 4.2). Average lupine N_2 fixation amounted to about one-third of N_2 fixed by cut grass-clover. Lupine N yield, representing the flexibly deployable N fraction, ranged between 98 and 157 kg ha^{-1}, averaging 117 kg ha^{-1}. Striking varietal differences in crop growth parameters including N yield and N_2 fixation were observed in Exp. I, where ‘Boruta’ was subjected to severe pigeon feeding damage. Nitrogen harvest index, i.e. the amount of N in lupine seed yield expressed as percentage of total N uptake, differed between the two varieties being significantly higher for ‘Boruta’ compared to ‘Azuro’, but was unaffected by the experimental year (Table 4.3).

Total N concentration of seeds averaged 5.2% and was neither affected by variety nor by the experimental year.

Table 4.3 Nitrogen harvest index (NHI) of lupine precrops and total N concentration (N_t) of lupine seeds. Mean values of experiment I and II. Different letters indicate significant differences between precrops at p≤0.05 (Fisher's LSD, n=3).

	Precrop	NHI (%)	N_t (%)
	Lupine 'Boruta'	60.4 a	5.3
	Lupine 'Azuro'	53.4 b	5.1
	Exp. I	56.2	5.2
	Exp. II	57.5	5.2
ANOVA	Precrop	*	NS
	Exp.	NS	NS
	Precrop x Exp.	NS	NS

NS, * = non-significant or significant at p≤0.05, respectively.

4.3.4 Available N for white cabbage

Grass-clover

The net amount of N mineralized from grass-clover residues during cabbage growth varied between 46 and 127 kg ha^{-1}, which corresponds to a range of 26 to 42% of the N amount incorporated with grass-clover residues (Table 4.4). Residue net N mineralization was strongly positively related to the content of clover in the mixture (Fig. 4.1b). Spring SMN under grass-clover swards was increased by mulching compared to cutting in Exp. I by 55 kg ha^{-1} (Table 4.4), which were largely recovered in the upper 30 cm of the soil profile (Fig. 4.2). In contrast, sward management had no effect on spring SMN in Exp. II, averaging 28 kg ha^{-1}.

Total available N for cabbage was considerably affected by sward management in Exp. I amounting to 320 kg ha^{-1} for mulched and 233 kg ha^{-1} for cut swards (Table 4.4). In Exp. II, cabbage N_{av} averaged 210 kg ha^{-1} over both management treatments, which was less than cabbage N_{av} after spring wheat.

Table 4.4 Total amount of plant available N (N_{av}) for white cabbage grown in the year subsequent to lupines, mulched (MU) and cut (CT) grass-clover and spring wheat as composed of spring soil mineral N (SMN) in 0-120 cm soil depth, net N mineralization from soil organic matter (SOM) and from incorporated legume biomass, i.e. lupine coarse meal (CM) and grass-clover residues respectively

	Precrop	N_{av}	SMN	SOM net N mineralization	Legume net N mineralization	
		kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	%	kg ha^{-1}
Exp. I	Lupine 'Boruta'	288.9	112.7	108.2	71.7^{y}	68.0^{w}
	Lupine 'Azuro'	342.8	130.1	96.6	72.8^{y}	116.1^{w}
	Grass-clover MU	319.8	107.4	85.1^{z}	39.4^{x}	127.4
	Grass-clover CT	232.5	52.8	85.1^{z}	41.7^{x}	94.6
	Spring wheat	187.0	101.9	85.1	-	-
Exp. II	Lupine 'Boruta'	382.8	223.8	97.7	53.8^{y}	61.3^{w}
	Lupine 'Azuro'	363.6	208.4	105.8	45.9^{y}	49.3^{w}
	Grass-clover MU	217.5	24.0	125.1^{z}	25.5^{x}	68.3
	Grass-clover CT	202.2	31.5	125.1^{z}	35.6^{x}	45.7
	Spring wheat	284.5	159.4	125.1	-	-
ANOVA	Precrop	***	***	NS	NS	NS
	Exp.	NS	***	NS	NS	NS
	Precrop x Exp.	*	***	NS	NS	NS
Fisher's LSD (p≤0.05)	Precrop, Exp. I	94.1	30.1			
	Precrop, Exp. II	85.9	33.3			

NS, *, *** = non-significant or significant at p≤0.05 or p≤0.001, respectively; n=3.
^{z}Assuming SOM net N mineralization of non-leguminous precrop (spring wheat).
^{y}Calculated as difference between amounts of N mineralized from plots amended with lupine coarse meal and unamended control plots. Expressed as percentage of N applied with coarse meal (180 kg ha^{-1}).
^{x}Calculated as difference between amounts of N mineralized from plots precropped with spring wheat and grass-clover. Expressed as percentage of N in grass-clover residues (Table 4.2).
^{w}Corrected for the amount of lupine N yield (LNY) harvested in the previous year (Table 4.):
CM net N mineralization (kg ha^{-1}) = LNY (kg ha^{-1}) × CM net N mineralization (%) × 0.01

Lupine

Reallocation of LNY as coarse milled seed meal contributed between 49 and 116 kg ha^{-1} to potential cabbage N_{av} depending on the magnitudes of LNY and net N mineralization from lupine coarse meal (Table 4.4). The percentage of net N mineralized from coarse meal ranged between 46 and 73%, averaging 61%, and was higher than net N mineralization from grass-clover residues. Spring SMN after lupines was generally higher than after grass-clover swards, particularly when swards had been cut. While in Exp. I, spring SMN after lupines was largely recovered in 0-60 cm soil depth, considerable amounts of on average 102 kg ha^{-1}

were found in soil layers between 60 and 120 cm in Exp. II (Fig. 4.2). Potential cabbage N_{av} of lupine treatments averaged 344 kg ha^{-1} and was in Exp. I comparable to cabbage N_{av} resulting from mulched grass-clover swards (Table 4.4). Apart from that, cabbage N_{av} of lupine treatments was found to be higher than N_{av} after grass-clover.

The precrop N effect of lupines on cabbage N_{av} was neither affected by lupine genotype nor by the experimental year, averaging 35 kg N ha^{-1} (Table 4.5). This corresponds to, on average, 38% of the N amount in lupine crop residues. The total N benefit of lupine treatments for white cabbage as composed of precrop N effect and net mineralized N from harvested and reallocated seeds ranged between 83 and 151 kg N ha^{-1}. From this, it can be derived that lupine seed N contributed between 60 and 77% to the total N benefit for cabbage, averaging 66%.

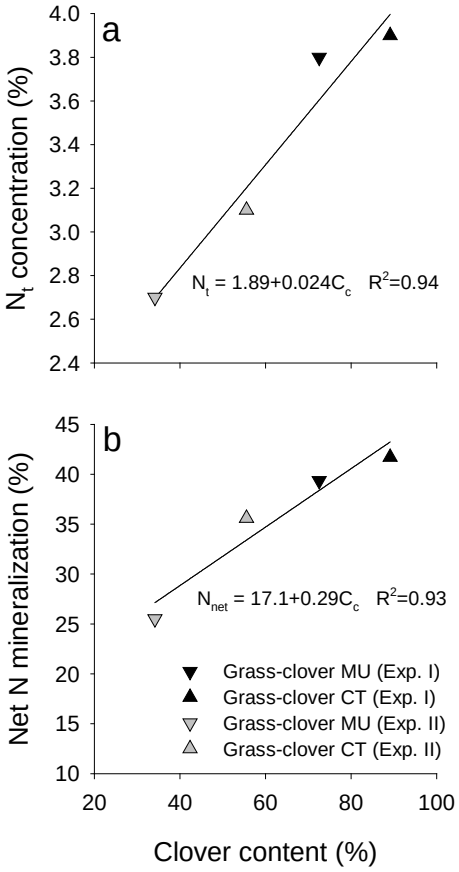

Fig. 4.1 Dependencies of total N concentration (N_t) in above-ground grass-clover biomass at incorporation (a) and net N mineralization (N_{net}) from grass-clover sward residues within the first year after incorporation (b) on the content of clover in living above-ground sward dry matter (C_c). MU, CT = mulched and cut swards, respectively.

4.3.5 Residual N effects

Total available N for beetroot was highest after mulched grass-clover and lowest after wheat, while lupine treatments and cut grass-clover were intermediate (Table 4.6). As residual SMN at cabbage harvest was not affected by precrop type, differences in beetroot N_{av} were due to variation in net N mineralization from cabbage residues and from SOM. The residual N effect of lupine treatments, i.e. the increment in SOM mineralization between cabbage and beetroot harvest in reference to spring wheat, amounted to an average of -2 kg ha^{-1}. Positive residual

N effects were found after grass-clover amounting to 24 kg ha^{-1} for mulched and 32 kg ha^{-1} for cut swards, which corresponds to 7 and 14% of the N amount incorporated with grass-clover residues, respectively. However, differences in residual N effects between legume precrops were not statistically evident.

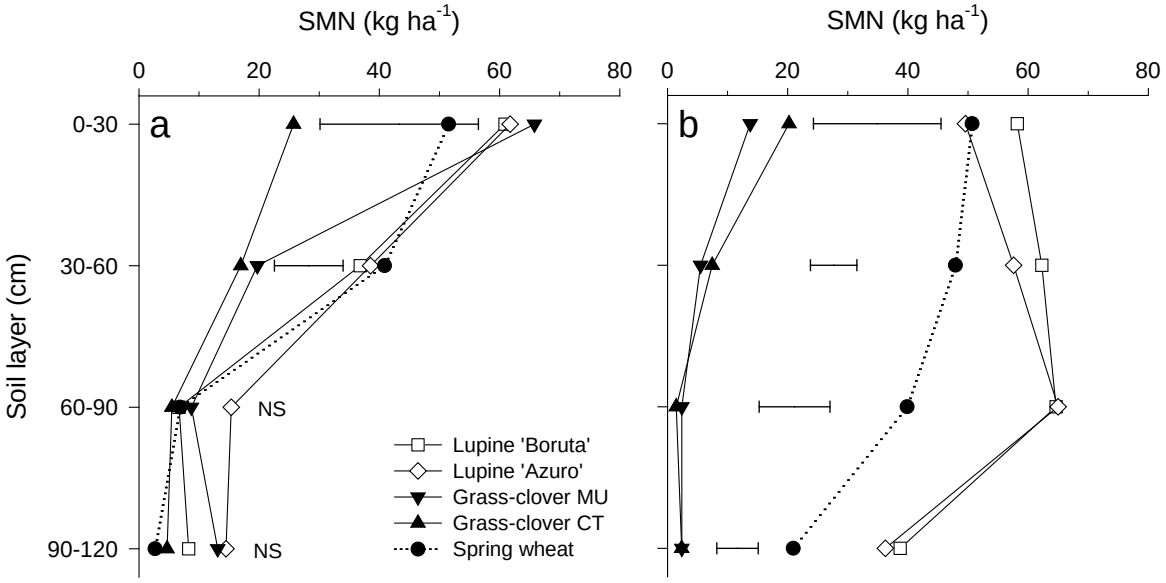

Fig. 4.2 Spring soil mineral N (SMN) after different precrops as a function of soil depth for experiment I (a) and II (b). Horizontal bars represent least significant differences between precrops (Fisher's LSD, p≤0.05, n=3).

Table 4.5 Precrop N effect of lupines (PE_{lup}) on N availability for succeeding white cabbage

	Precrop	PE_{lup} (kg N ha^{-1})
	Lupine 'Boruta'	35.5
	Lupine 'Azuro'	34.8
	Exp. I	36.9
	Exp. II	33.5
ANOVA	Precrop	NS
	Exp.	NS
	Precrop x Exp.	NS

NS = non-significant; n=3.

Table 4.6 Amount of total available N (N_{av}) for beetroot as composed of residual soil mineral N in 0-120 cm soil depth present in previous autumn at cabbage harvest (SMN), net N mineralization from cabbage residues (CR) and from soil organic matter (SOM) for lupines, mulched (MU) and cut (CT) grass-clover and spring wheat and residual N effect of respective legume precrops (RE_{leg})

Precrop	N_{av}	SMN	CR net N mineralization[z]	SOM net N mineralization	RE_{leg}[y]
			kg ha^{-1}		
Lupine 'Boruta'	235.2	40.6	88.8	105.8	-10.5
Lupine 'Azuro'	258.2	37.9	98.1	122.2	5.9
Grass-clover MU	281.1	44.4	96.7	139.9	23.7
Grass-clover CT	254.9	38.9	67.8	148.2	32.0
Spring wheat	203.2	40.3	46.6	116.3	-
Fisher's LSD (p≤0.05)	58.3	NS	22.5	NS	NS

NS = non-significant; n=3.
[z]Calculated as 63% of the N amount in cabbage residues.
[y]Increment in SOM net N mineralization in reference to spring wheat.

4.3.6 N budgets

Grass-clover

Mulching of swards resulted in a substantial surplus in precrop N balance of up to 225 kg ha^{-1}, while the precrop N balance of cut swards did not exceed +82 kg ha^{-1}, averaging -4 kg ha^{-1} (Table 4.7). Simple N balances of grass-clover based crop rotations averaged +9 and -85 kg ha^{-1} after harvest of white cabbage for mulched and cut swards, respectively.

Lupine

Precrop N balance of lupines ranged from -22 to +55 kg ha^{-1}, averaging +2 kg ha^{-1} (Table 4.7), i.e. on average of both experiments and varieties the amount of N in lupine seed yield equaled approximately the amount of N_2 fixed. Reallocation of lupine seed N in amounts of 180 kg ha^{-1} corresponded to 115 to 183% of the N amount previously harvested with lupine seeds.

Simple N balance after white cabbage averaged +31 kg ha^{-1} (Table 4.7). Although in Exp. II simple N balance was found to be significantly higher for 'Azuro' than for mulched grass-clover, there was no substantial overall difference in N balance

between lupine and mulched grass-clover. After beetroot harvest, all investigated legume systems showed negative simple N balances.

Table 4.7 Precrop N balance, additional N input by lupine coarse meal, N output due to harvested cabbage and beetroot biomass and resulting simple N balance after cabbage and after beetroot for lupines and mulched (MU) and cut (CT) grass-clover

	Precrop	Precrop N balance	N input[z]	N output		Simple N balance	
			Lupine coarse meal	Cabbage N yield	Beetroot N yield	Precrop + Cabbage	Precrop + Cabbage + Beetroot
		kg ha^{-1}					
Exp. I	Lupine 'Boruta'	-12.6	180 (179%)	123.0	139.6	44.3	-95.2
	Lupine 'Azuro'	55.0	180 (115%)	126.7	144.8	108.3	-36.5
	Grass-clover MU	224.8	-	117.8	150.9	107.0	-43.9
	Grass-clover CT	82.0	-	83.4	149.6	-1.4	-151.0
Exp. II	Lupine 'Boruta'	-21.9	180 (159%)	187.2	-	-29.1	-
	Lupine 'Azuro'	-11.0	180 (183%)	169.1	-	-0.1	-
	Grass-clover MU	1.5	-	89.6	-	-88.1	-
	Grass-clover CT	-89.4	-	79.2	-	-168.6	-
ANOVA	Precrop	***		***	NS	***	*
	Exp.	***		*	-	***	-
	Precrop x Exp.	**		**	-	*	-
Fisher's LSD (p≤0.05)	Precrop, Exp. I	84.8		NS	NS	80.2	87.7
	Precrop, Exp. II	NS		34.0		60.6	

NS, *, *** = non-significant or significant at p≤0.05 or p≤0.001, respectively; n=3.
[z]Values in brackets represent the N input by lupine coarse meal expressed as percentage of lupine N yield of the previous year.

4.4 Discussion

4.4.1 N_2 fixation of legume precrops

It is well-established that symbiotic N_2 fixation of legumes is inhibited by high concentrations of inorganic soil N (Drevon et al. 1988; Leidi and Rodriguez-Navarro 2000; McAuliffe et al. 1958; Streeter and Wong 1988). The difference in average N_2 fixation between the two experiments is therefore likely to be explained by differences in indigenous soil N supply. The low-alkaloid lupine variety 'Boruta' appeared to deviate from this dependency, showing similarly low

N_2 fixation estimates in both experiments. The reason for the comparatively weak performance of 'Boruta' in Exp. I is to be found in severe pigeon feeding damage, which limited plant growth and consequently N_2 fixation. The high-alkaloid variety 'Azuro' was unaffected by the occurrence of birds, pointing out the role of alkaloids as feeding deterrents against several kinds of herbivores (Wink 1983).

Except for lupine crops damaged by bird feeding, N_2 fixation of lupines approximated that of mulched grass-clover swards but was largely outperformed by grass-clover if cut herbage was removed. The higher N_2 fixation potential of grass-clover compared to lupines is likely to be related to the longer growth period, allowing for additional N_2 fixation in the period between lupine harvest in early autumn and sward ploughing in subsequent spring. Remaining grass-clover mulch decreased N_2 fixation compared to removal of cut herbage by on average 185 kg ha^{-1}, which is consistent with previous studies reporting a negative response of N_2 fixation by grass-clover to mulching (Dreymann 2005; Hatch et al. 2007; Loges et al. 2000). Mulch material remaining on the sward may affect N_2 fixation by means of both (i) covering the sward surface and thereby reducing light interception and clover productivity and (ii) increasing the content of soil mineral N due to mineralization of mulch N. An increase in soil mineral N constrains N_2 fixation directly via reducing nitrogenase activity and, as a second mechanism, indirectly via causing the percentage of clover in the mixture to decline by favoring growth of the companion grass (Høgh-Jensen 1997). The observed variation in clover content, ranging from 34 to 89%, can therefore largely be ascribed to differences in plant available soil N varying with experimental year and sward management treatment.

4.4.2 Available N for succeeding vegetable crops

Present results reveal that one-year-old grass-clover swards have the potential to provide sufficient N even to an N demanding vegetable crop grown subsequent to sward incorporation (Exp. I). However, grass-clover was also shown to provide less N than a non-leguminous precrop, even though N in cut herbage was for the most part not exported from the sward but remained in mulch material (Exp. II). Variation in the preceding crop effect of grass-clover swards is frequently

explained by the N effect of ploughed-in sward residues, i.e. by their amount and quality (Dreymann 2005; Høgh-Jensen and Schjoerring 1997; Loges 1998). In the present study, mulching increased the amount of N in residues considerably by 98 kg ha^{-1} but lead to a significant reduction in the proportion of clover. Low clover content, in turn, was accompanied by low N_t concentration and low net N mineralization of sward residues. Similarly, Loges (1998) reported that wheat N uptake increased with clover content of a preceding grass-clover sward and Nykänen et al. (2008) found a positive relationship between the C:N ratio of incorporated grass-clover biomass and subsequent cereal yield. However, the lower N availability after grass-clover compared to spring wheat in Exp. II was not solely explained by a low net mineralization of sward residue N but was rather due to a considerable difference in spring soil mineral N, averaging 28 kg ha^{-1} for grass-clover and amounting to 159 kg ha^{-1} for spring wheat. According to our observations, under low leaching conditions, Willumsen and Thorup-Kristensen (2001) reported lower spring soil mineral N and lower white cabbage yield following winter-persistent grass-clover compared to winter-killed green manures or bare soil. Yield reduction in cabbage was attributed to a 'pre-emptive competition' for N between grass-clover and cabbage plants. In addition, present results show that spring soil mineral N is affected by sward management. Mulching increased spring soil mineral N by 55 kg ha^{-1} (Exp. I), indicating that a considerable proportion of N in mulched grass-clover herbage was mineralized before sward ploughing. The effect of mulching on spring soil mineral N is likely to be affected by (i) the amount of N in mulched herbage, (ii) mulch N_t concentration and (iii) re-uptake of mineralized mulch N and, thus, depends to a large extend on the percentage of clover. This would explain the difference in the response of spring soil mineral to mulching between Exp. I and II. Thus, on soils with high water-holding capacity being not nitrate-depleted in spring by drainage, the preceding crop effect of grass-clover swards is not entirely explained by the N effect of ploughed-in sward residues. Under these conditions, the amount of soil mineral N present in spring can vary considerably with sward history, i.e. sward performance and management practices.

Contrary to grass-clover, the precrop N effect of lupines was strikingly similar between experimental years as well as between lupine genotypes. The average lupine precrop N effect of 35 kg N ha^{-1} corresponded to 38% of the N amount in residues, which is in remarkable agreement with results obtained by Russell and Fillery (1999) investigating the N mineralization from individual components of ^{15}N-labeled lupine (*L. angustifolius* L.) residues under field conditions. The annual net mineralization of N in residual leaf, stem and pod biomass amounted to 42%. From this, an N benefit from residues of a typical lupine crop of 25 to 42 kg ha^{-1} was estimated. Fink (2000) reported an N benefit from pea (*Pisum sativum* L.) residues to a succeeding spinach crop of 30 kg ha^{-1}, corresponding to a net N mineralization of 31%. Similarly, on a loam soil, the N uptake of winter barley following lupine and pea was found to be 18 to 27 kg N ha^{-1} higher than following oat (Jensen et al. 2004).

According to our expectations, the amount of N_2 fixed by lupines approximated, on average, the N amount in seed yield and the N_t concentration of seeds was stable over the range of experimental conditions. Net N mineralization of reallocated, coarsely milled seeds averaged 61%, which demonstrates their high N fertilizer value. Furthermore, about two-third (60-77%) of the total N benefit of lupine treatments was assigned to the N fertilizer effect of coarsely milled seeds, which can be managed flexibly in time and space. However, lupine treatments did not show a residual N effect on beetroot grown subsequent to cabbage, indicating that net N mineralization from both lupine residues and coarse meal was largely completed at cabbage harvest.

In contrast to the well-controllable but rather short-lived N benefit of lupine treatments, grass-clover showed a considerable residual N effect of on average 28 kg ha^{-1}, which corresponds to 11% of the N amount in ploughed-in grass-clover residues. This agrees well with results obtained by Laber (2007) investigating the N benefit from differently managed, two-year-old (grass-)clover and alfalfa (*Medicago sativa* L.) swards. The residual N effect on a second crop after sward incorporation was largely unaffected by sward management, averaging 30 kg ha^{-1} (±3.8 SEM, n=13). Studies in which ^{15}N-labeled root and shoot material of (grass-)clover and alfalfa was soil incorporated in defined

amounts revealed comparatively lower residual N effects on a second crop, amounting to only 1.2 to 2.8% of applied legume N (Harris and Hestermann 1990; Haynes 1997; Wivstad 1999). The residual N effect of grass-clover swards might therefore rather be due to soil organic N, which is built up during the period of sward growth by N rhizodeposition (Høgh-Jensen and Schjoerring 2001), than to late mineralizing sward residue N. The two major rhizodeposition pathways are decomposition of roots and nodules and exudation of N-rich compounds by plant roots (Fustec et al. 2010; Wichern et al. 2008).

4.4.3 Legume below-ground N

By focusing solely on above-ground N of legumes, their N_2 fixation and contribution to N budgets of legume-based crop rotations may be substantially underestimated (Huss-Danell et al. 2007; Peoples et al. 2009). In the present study, below-ground N was accounted for by using literature reported root/shoot ratios obtained by conventional root excavation and separation-washing. As a considerable proportion of fine roots is typically lost during the washing process and also rhizodeposits are not recovered, below-ground N quantified by root excavation methods represents rather conservative estimates. Recoverable root N of *L. angustifolius* was reported to amount to 35% of total lupine below-ground N quantified by means of ^{15}N-labeling (Russell and Fillery 1996). For *L. albus*, a respective proportion of 15% can be derived from data presented by Mayer et al. (2003) using the same methodology. In turn, 85% of total below-ground N was not recovered in lupine roots and was defined by (Mayer et al. 2003) as rhizodeposition N. Relating these data to the present study, lupine below-ground N would have been underestimated by 17 to 87 kg N ha^{-1}, largely depending on the proportion of rhizodeposition N to total below-ground N chosen. This N amount corresponds to, on average, 62% of lupine residue N presented in Table 4.3.

Nitrogen rhizodeposits of grain legumes have been either shown to mineralize to 21 to 30% within periods of 3 to 5.5 months (Jensen 1996; Mayer et al. 2004) or appeared to form a SOM pool with high decomposability (Mayer et al. 2003). They are therefore widely assumed to play an important role in the N dynamics after

grain legumes. This is not supported in the present study, in which the precrop N effect of lupines was reasonably well explained by the N amount in lupine residues and residual N effects on beetroot grown as a second crop after lupines were not observed. Thus, if N rhizodeposition by lupines should have contributed to the N supply for cabbage and beetroot, the magnitude of this effect must have been similar to that of spring wheat used as reference to quantify precrop and residual N effects. The particular importance of N rhizodeposition by grain legumes as N source for subsequent crops can therefore be questioned.

The proportion of N located in the root biomass of grass-clover swards is typically higher than that of grain legumes at crop maturity. Particularly if cut herbage is removed from the sward, grass-clover residues consist to a large proportion of below-ground biomass. Thus, the contribution of below-ground N of grass-clover swards to available N for subsequent crops can be substantial. Net N mineralization from clover and ryegrass roots was shown to amount to 25 to 30% of soil-added root N after time periods of 4.5 to 8.5 months (Bending et al. 1998; Frankenberger and Abdelmagid 1985; Wivstad 1999). In the present study, the apparent net N mineralization from grass-clover residues, consisting of root, stubble and (mulched) herbage biomass, varied between 26 and 42% after 4.5 months. Even though these mineralization data cannot be compared directly, it suggests that grass-clover root N was not severely underestimated by using root/shoot ratios obtained by conventional root excavation methods. However, residual N effects of ploughed-in grass-clover swards indicate a large discrepancy between total grass-clover below-ground N including rhizodeposits and root N being recoverable by excavation.

4.4.4 N leaching potential

Even though drainage to below 120 cm soil depth did not occur in the present study, the risk for N losses due to leaching is indicated by the amount and distribution of soil mineral N present in spring. From this, cut grass-clover can be identified as the precrop with the lowest N leaching potential. Mulching increased spring soil mineral N either only in the upper 30 cm of soil (Exp. I) or not all (Exp. II) and thus increased the N leaching potential only moderately. On sandy

soils, however, mulching of grass-clover swards was shown to cause N leaching losses of considerable magnitude (Dreymann 2005).

Assuming the scenario that spring soil mineral N recovered in 60-120 cm soil depth would have been drained to below 120 cm, leaching losses after lupines would have averaged 22 kg N ha^{-1} in Exp. I and 102 kg N ha^{-1} in Exp. II, while corresponding losses for mulched grass-clover swards would have amounted to 22 and only 4 kg N ha^{-1}. Variation in potential N leaching after lupines between experiments indicates that N leaching after lupines is positively associated with indigenous soil N supply during lupine crop growth. Thus, the substantial difference in potential N leaching between mulched grass-clover and lupine precrops (Exp. II) can be largely ascribed to (i) elevated residual soil mineral N at lupine harvest (Chalk et al. 1993; O'Connor et al. 2009) and in return to (ii) the uptake of soil mineral N mainly by the grass component of grass-clover mixtures (Dreymann 2005). In addition, mineralization of lupine root N and N rhizodeposits after crop harvest (Mayer et al. 2004) as well as of above-ground residues might to some extent contribute to N leaching during the following percolation period.

4.5 Conclusions

Variation in the percentage of clover in grass-clover swards contributes greatly to uncertainty in N supply for vegetable crops cultivated after sward ploughing. Removal of cut grass-clover herbage leads to a considerable increase in both clover content and N_2 fixation. However, due the export of N with removed herbage, the N benefit of cut swards for subsequent crops is unsatisfactorily low.

Compared to mulched grass-clover swards, the local production of lupine seeds followed by their reallocation as fertilizer represents a flexible N source alternative, which allows for uncoupling N availability for organically grown vegetable crops from the performance of the preceding legume green manure. Two principle differences between grass-clover and lupine are to be considered. Firstly, at high levels of plant available N during lupine crop growth, large amounts of mineral N accumulate in the soil after crop harvest, which are potentially lost by leaching. In contrast, grass-clover mixtures respond to high N availability with an

elevated percentage of the grass component, leading to a substantial reduction in potential N leaching. Secondly, the N benefit from lupine crop residues and lupine coarse meal is rather short-lived, while ploughing of grass-clover swards has a positive longer-term effect on net N mineralization from SOM.

5 Final discussion

Besides restrictions in the control of pests, diseases and weeds, shortage of plant available N is the leading cause for lower crop yields in organic compared to conventional vegetable production. The use of leguminous green manure crops can provide a sizeable import of N into the system via fixation of atmospheric N_2. However, as their cultivation occupies a significant proportion of the crop rotation, the yield gap between organic and conventional management is even greater if the production systems are compared on a *crop rotation basis* than on a *crop basis*. Approaches to narrowing the yield gap need to gain as much benefit from legume green manures as possible by maximizing N_2 fixation and increasing the efficiency with which fixed N_2 is utilized within the crop rotation. A crucial factor for an efficient use of legume derived N is a high *degree of flexibility*, allowing N supply to match crop demand.

5.1 Aspects of maximizing N_2 fixation

Legume-based crop rotations aiming to be self-sufficient in N require that N_2 fixation compensates for the amount of N exported with crop yield and diffuse losses. As indicated by simple N balances, the N output during two years of vegetable production was not compensated by one preceding year of legume cultivation, irrespective of legume type and management practices (Chapter 4). Since the share of green manure legumes in the crop rotation is limited by economic constraints, the N input via symbiotic N_2 fixation needs to be maximized without reducing vegetable crop production to below the economic threshold.

Organic systems rely to a large extent on N deriving from mineralization of indigenous soil organic N. It is therefore aimed to build up the soil organic matter pool, which supplies mineral N for crop growth. However, symbiotic N_2 fixation directly interacts with soil N status, being up-regulated when soil mineral N is low and being down-regulated when soil mineral N is high. Thus, increasing the ability of a soil to supply N via mineralization during the growing season is doing a

disservice to N_2 fixation, implying a principle contradiction within organic farming. Managing the N supply for legume crops can therefore be considered as keystone for maximizing the input of atmospherically derived N into organic agro-ecosystems.

Tillage causes soil disturbance, accelerating N transformation processes and thereby increasing crop N supply. A reduction in tillage intensity may therefore be an effective strategy for increasing legume N_2 fixation. When compared to conventional plough treatments, reduced tillage has been shown to increase N_2 fixation of soybean (Wheatley et al. 1995), pea and red clover (Reiter et al. 2002). However, since moldboard ploughing plays an important role in the suppression of weeds, a continuous abandonment of ploughing will be problematic in organic vegetable production at most locations.

When incorporated into the soil, organic materials with high C:N ratio may reduce crop N availability by means of immobilization of soil mineral N. Straw applications were reported to enhance N_2 fixation of subsequent pea (Evans et al. 1997), fenugreek (Abd-Alla and Omar 1997) and soybean (Shivashankar and Vlassak 1978), which is not exclusively ascribed to the altered N availability but additionally to an increase in CO_2 assimilation activity of root nodules (Fischinger and Schulze 2010).

However, the increase in N_2 fixation by means of these practices can be expected to be small compared to the influence of sward management on N_2 fixation of grass-clover mixtures. Mulching is common practice in stockless organic production systems, even such practice decreases N_2 fixation and does not exploit the full potential of grass-clover swards. If cut herbage was removed from the field, N_2 fixation of spring-ploughed grass-clover was shown to exceed that of lupine by more than twofold (Chapter 4). Dinitrogen fixation of white clover determined by *in situ* measurement of acetylene reduction activity is reported to occur throughout the year except at snowfall (Sugawara and Izawa 1995) and to increase substantially if soil temperature exceeds 3°C (Marriott 1988). Under Danish climatic conditions, the average daily N_2 fixation rate of clover grown in pure stand varied from 0.5 kg N ha^{-1} day^{-1} in autumn to 3.3 kg N ha^{-1} day^{-1} in June (Jørgensen et al. 1999). Thus, the amount of N_2 fixed by perennial forage

legumes during autumn and spring can be of considerable magnitude, while N_2 fixation activity of grain legumes typically terminates at pod formation stage (Carranca et al. 2009; Kyei-Boahen et al. 2002; Peña-Cabriales et al. 1993). In terms of maximizing N_2 fixation, the extended growing period of perennial forage legumes represents a substantial advantage compared to grain legumes. This is particularly the case for vegetable production systems in which the soil is usually uncropped during winter, and thus growing legumes as N source does not compete with vegetable crop production.

5.2 Further prospects of 'mobile green manuring'

Within the investigated legume systems only lupine allowed for uncoupling N availability for vegetable crops from the performance of the preceding legume (Chapter 4). The amount of N in lupine seeds is well storable, highly mobile and was found to possess a controllable and comparatively high fertilizer effect if seeds are reallocated as a coarse meal (Chapter 2 and 3). Consideration of forage legume systems including the return of cut herbage N was beyond the scope of the present study. However, higher N_2 fixation and a lower potential for N leaching losses of cut grass-clover swards compared to lupine (Chapter 4) raises the question of a mobile utilization of cut herbage N. Freshly cut sward herbage might be directly transferred to vegetable fields and either incorporated before crop establishment or applied to already established crops as surface mulch. Net N mineralization of red clover herbage being surface applied to white cabbage and leek crops was found to be low, ranging from 4 to 26% (Båth and Elfstrand 2008; Båth et al. 2003). Correspondingly, the apparent recovery of (grass-)clover mulch N in above-ground biomass of beetroot and white cabbage was reported to average only 11% with maximum values not exceeding 27% (Riley et al. 2003). In addition, since cut grass-clover herbage is hardly storable, herbage production would have to be in synchrony with vegetable production, creating serious uncertainty in crop N supply. This disadvantage also applies to soil incorporation of freshly cut grass-clover herbage, even though a higher proportion of up to 50% can be expected to become plant available (Chapter 4; Breland 1994; Laber 2007).

Alternatively, grass-clover herbage might be fermented using anaerobic digester for biogas production. Besides additional energy yield, biogas digestate can be flexibly reallocated within the crop rotation to non-legume crops. Furthermore, biogas digestion might economically justify the removal of *Brassica* residues from the field. This would have positive impact on N use efficiency by reducing potential N leaching losses associated with N-rich vegetable residues (Chapter 3) as well as by making residue N flexibly available. Field studies in which liquid effluent from biodigested plant material was applied as fertilizer revealed either positive (Gunnarsson et al. 2010; Stinner et al. 2008) or insignificant (Båth and Elfstrand 2008; Ross et al. 1989) yield effects. Differences in the extent of ammonia volatilization caused by different application techniques and conditions probably contributed greatly to the variability of N fertilizer value of liquid digestate, and thus will have decisive influence on N use efficiency and advisability of such a cropping system. Möller et al. (2010) estimated total losses during storage and spreading of previously solid-liquid separated biogas digestates, mainly deriving from silage maize and grass, to amount to 20 to 25%. Losses of this magnitude would severely diminish the N_2 fixation benefit of cut grass-clover compared to lupine and would also have to be viewed critically from the environmental point of view. Furthermore, due to a minimum water content of 90%, total N concentration in the fresh matter of unprocessed liquid digestate is typically well below 5 g l^{-1} (e.g. Båth and Elfstrand 2008; Gunnarsson et al. 2010; Stinner et al. 2008). Thus, to increase its transportability, to solve storage problems as well as to avoid soil compaction during application, the volume of liquid digestate might be required to be reduced by dehydration (Maurer and Müller 2010; Möller et al. 2010). Besides additional energetic expense, sophisticated dehydration systems require additional investment and operating costs and may therefore often not be feasible for local on-farm digester of limited size.

Contrary to on-farm digestion with the primary aim of increasing the flexibility of legume N, digestate deriving as by-product from centralized biogas plants might play an increasing role as nutrient source in future vegetable production.

However, this would not contribute to the objective of closing nutrient cycles and developing organic vegetable rotations being self-sufficient in N.

5.3 Closing remarks

The present study provides proof-of-concept that lupines, integrated into organic vegetable rotations, serve as flexible N source alternative to traditional forage legumes. When putting this approach into practice, site- and farm-specific realities as well as economic aspects have to be considered, ultimately deciding on its acceptance by vegetable producers.

Looking back, the aim of closed nutrient cycles has always been a key component in the self-conception of organic farming. This objective is presently intended to be ensured by different organic standards, setting the boundary of the organic production system as well as regulating the import of N from external sources. Future interest in and demand for utilizing legumes as local N source, in general, are associated with future revisions of these standards and how far they will depart from initial principles of organic farming. Accordingly, the ratio of producers being member of organic agriculture associations with comparatively strict standards to those following less strict standards like the EU regulation 834/2007 will have major impact on this development.

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Erklärung zur Dissertation

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Hierdurch erkläre ich, dass ich meine Dissertation mit dem Titel

“Narrow-leaved Lupine (*Lupinus angustifolius* L.) as Nitrogen Source in Organic Vegetable Production Systems”

selbständig verfasst und die benutzten Hilfsmittel und Quellen sowie gegebenenfalls die zu Hilfeleistungen herangezogenen Institutionen vollständig angegeben habe.

Die Dissertation wurde nicht schon als Masterarbeit, Diplomarbeit oder andere Prüfungsarbeit verwendet.

Kai-Uwe Katroschan